(54) Title: SCAFFOLD-FREE SELF-ORGANIZED 3D SYNTHETIC TISSUE

(57) Abstract: The present invention can be used for actual implantation surgery without a scaffold. The present invention provides
a synthetic tissue or complex, which can be produced by culture and has a high level of differentiation ability. The present invention
also provides a therapy and medicament for requiring and/or regenerating tissue using regeneration and covering. By culturing cells
under specific culture conditions such that medium contains an extracellular matrix synthesis promoting agent, the cells are organized
and are easily detached from a culture dish. The present invention was achieved by fixing such a phenomenon. In addition, the
self contraction of the tissue can be regulated by culturing the tissue in a suspended manner. Therefore, it is possible to regulate the
three dimensional shape of the tissue. The present invention also provides a method for producing an implantable synthetic tissue
which does not require a plurality of monolayer cell sheets assembled to form a three-dimensionally structured synthetic tissue. The
present invention is characterized by richness in adhesion molecules, nonnecessity of additional fixation at an implantation site, and
good biological integration.

WO 2005/012512 A1

DESCRIPTION

SCAFFOLD-FREE SELF-ORGANIZED 3D SYNTHETIC TISSUE

TECHNICAL FIELD

The present invention relates to the field of regenerative medicine. More particularly, the present invention relates to a synthetic tissue capable of functioning after implantation, a method for producing the same, and use of the same. The synthetic tissue of the present invention has biological integration capability.

BACKGROUND ART

Recently, regenerative therapy has attracted attention as a novel approach to severe organ failure or intractable diseases. Regenrative therapy is a combination of genetic engineering, cell tissue engineering, regenerative medicine, and the like. Many researchers over the world are vigorously working on this important and challenging subject of research in the 21-century advanced medical practice.

The scale of the market associated with regenerative medicine (tissue engineering) is estimated as about 500 billion US dollars in the world and about 50 billion US dollars in Japan according to the material prepared by the New Energy and Industrial Technology Development Organization. Only tissue engineering products account for about 100 billion US dollars in the world. The regenerative medicine is greatly expected to create the next-generation industry.

The present inventors have made efforts to develop regenerative therapy in the field of musculoskeletal and cardiovascular tissues, and have reported a combination therapy of cell implantation and a growth factor administration, or a tissue implantation regeneration therapy based on tissue engineering. However, regenerative therapy based on cell or tissue implantation requires a source of autologous cells. A stable and abundant source of such cells is urgently required and important. A number of cells in musculoskeletal tissue have a high level of self-repairing ability. It has been reported that there is a stem cell among the cells of the musculoskeletal tissue.

It has been demonstrated that a cell derived from skeletal muscle (Jankowiski R.J., Huand J. et al, Gene Ther., 9:642-647, 2002), fat (Wickham M.Q. et al., Clin. Orthop., 2003, 412, 196-212), umbilical cord blood (Lee O.K. et al., Blood, 2004, 103:1669-75), tendon (Salingcarnboriboon R., Exp. Cell. Res., 287:289-300, 2002), bone marrow (Pittenger M.F. et al., Science, 284:143-147, 1999), and synovium (Arthritis Rheum. 2001 44:1928-42) is undifferentiated and has the potential to differentiate into various cells.

Conventionally, when cell therapy is performed for repair or regeneration of tissue, most research employs a biological scaffold to maintain the accumulation of cells, allow cells to grow, maintain pluripotency, protect cells from mechanical stress on a treated site, or the like. However, most scaffolds contain a biological (animal) material, a biomacromolecule material, or the like, of which influence on the safety of organism cannot be fully predicted.

A cell implanting method without a scaffold has been

reported by Kushida A., Yamato M., Konno C., Kikuchi A.,
Sakurai Y., Okano T., J. Biomed. Mater. Res., 45:355-362,
1999, in which a cell sheet is produced using a temperature
sensitive culture dish. Such a cell sheet engineering
5   technique is internationally appraised due to its originality.
However, a single sheet obtained by this technique is fragile.
In order to obtain the strength that can withstand surgical
manipulation, such as implantation, a plurality of sheets
need to be assembled, for example.
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When a nano-biointerface technology is used to fix
a temperature responsive polymer (PIPAAm) onto a plastic
mold, such as a Petri dish, for cell culture, the polymer
surface is reversibly changed at 31°C between hydrophilicity
15   and hydrophobicity. Specifically, when the temperature is
31°C or more, the surface of the Petri dish is hydrophobic
so that cells or the like can adhere thereto. In this
situation, the cells secrete extracellular matrix (ECM; for
example, adhesion molecules which are proteins having a
20   function like a "glue") and adhere to the surface of the
Petri dish, so that the cells can grow. See, Okano T., Yamada
N., Sakai H., Sakurai Y., J. Biomed. Mater. Res., 1993,
27:1243-1251; Kushida A., Yamato M., Konno C., Kikuchi A.,
Sakurai Y., Okano T., J. Biomed. Mater. Res. 45:355-362,
25   1999; and Shimizu T., Yamato M., Akutsu T. et al., Circ.
Res., 2002, Feb 22; 90(3):e40.

When the temperature is 31°C or less, the surface
of the Petri dish is hydrophilic. The cells which have
30   adhered to the Petri dish are readily detached, though the
cells still maintain adhesion molecules. This is because
the surface of the Petri dish to which the cells have adhered
no longer exists at 31°C or less.

~ 4 ~

Even when such a Petri dish having a fixed temperature responsive polyer (e.g., tradename: UpCell and RepCell) is used to culture cells and detach the cells, an extracellular matrix is not appropriately provided. Thus, there has been no actually practical synthetic tissue developed. See, Okano T., Yamada N., Sakai H., Sakurai Y., J. Biomed. Mater. Res., 1993, 27:1243-1251; Kushida A., Yamato M., Konno C., Kikuchi A., Sakurai Y., Okano T., J. Biomed. Mater. Res. 45:355-362, 1999; and Shimizu T., Yamato M., Akutsu T. et al., Circ. Res., 2002, Feb 22; 90(3):e40.

WO00/51527 and WO03/024463 reported that cells are cultured on a semipermeable membrane using alginate gel. However, the resultant tissue is poorly integrated with an extracellular matrix and is not free of a scaffold. In addition, the cells in the tissue are not self organized. The tissue has no self-supporting ability. The cells no longer have a differentiation potential. The tissue loses morphological plasticity in terms of three-dimensional structure. Therefore, the tissue is not suitable for cell implantation.

Use of a scaffold is considered to be problematic in implantation therapy because of adverse side effects. Therefore, there is a demand for the advent of a scaffold-free technique.

Conventional methods for producing tissue sheets have the following drawbacks: it is not possible to produce a very large sized sheet; it is not possible to produce a sheet having biological integration in three dimensions; when a sheet is detached after sheet production, the sheet

is broken into pieces; and the like.

Therefore, there is a keen demand for a synthetic
tissue, which is developed by culture processes, capable
5   of withstanding an implantation operation, capable of being
used in an actual operation.

By conventional techniques, it is difficult to
isolate a synthetic tissue from a culture base material after
10   tissue culture, and it is substantially impossible to produce
a large sized tissue piece. Therefore, conventional
synthetic tissues, such as tissue sheets, cannot be used
in medical application in view of size, structure, mechanical
strength, and the like. It is difficult to develop a
15   synthetic tissue using conventional techniques. Therefore,
unfortunately their supplies are limited.

An object of the present invention is to provide a
synthetic tissue produced by cell culture, which is feasible
20   to implantation surgery.

Specifically, an object of the present invention is
to provide a synthetic tissue having a three-dimensional
structure and self-supporting ability, being free of a
25   scaffold, and maintaining a differentiation potential if
the tissue possesses it.

Still another object of the present invention is to
provide a method and a pharmaceutical agent for treating
30   an injury of a tissue or the like when a replacement or
resurfacing therapy is required.

DISCLOSURE OF THE INVENTION

~ 6 ~

The above-described objects were achieved in part based on the invention of the following synthetic tissue. When a cell was cultured in medium containing an extracellular matrix (ECM) synthesis promoting agent, cells and ECM produced by the cells are integrated to formed a tissue, which was readily detached from the culture dish.

The above-described objects were achieved by providing a synthetic tissue of the present invention which is free of a scaffold, has self-supporting ability, is easily formed into a three-dimensional structure, has morphological plasticity, has excellent ability to biologically adhere to surroundings, has a differentiation potential, and the like, and finding that the synthetic tissue is effective for a replacement or resurfacing therapy at an injured site.

The present invention also provides a method for producing an implantable synthetic tissue, which has biological integration and does not require assembling layers.

The above-described objects were achieved by finding that the thickness of the synthetic tissue of the present invention can be adjusted to a desired value by regulating a physical or chemical stimulus on the synthetic tissue.

The present inventors realized the formation of a three-dimensional synthetic tissue (cellular therapeutic system) comprising cultured cells (e.g., fat-derived cells, etc.) and material produced by the cells without a scaffold.

The synthetic tissue of the present invention can

be constructed into various shapes and has a sufficient
strength. Therefore, it is easy to surgically manipulate
(e.g., implant, etc.) the synthetic tissue of the present
invention. According to the present invention, a large
5   quantity (e.g., 10^6 to 10^8) of cells can be securely supplied
to a local site by means of tissue implantation.

In the matrix, cell adhesion molecules, such as
collagen (e.g., type I, type III), fibronectin, vitronectin,
10   and the like, are present in large amounts. Particularly,
the cell adhesion molecules are integrated throughout the
matrix.

Therefore, the tissue has excellent ability of
15   biologically adhesion to surroundings of the implanted site.
Thus, the synthetic tissue complex biologically adheres to
an implanted site tissue very quickly. In addition, by
changing culture conditions, the synthetic tissue can be
differentiated into a bone or cartilage tissue. The
20   maintenance of a differentiation potential is a feature of
the synthetic tissue of the present invention which was first
found by the present inventors. The synthetic tissue is
effective as a safe and efficient cell therapy system.

25   An object of the present invention is to provide a
clinical application of the synthetic tissue regeneration
of a joint tissue. The present invention provides the
above-described synthetic tissue or a complex of a cell and
a component derived from the cell, thereby making it possible
30   to develop therapies for bone regeneration at a
conventionally intractable site, in which both periosteum
and bone cortex are inflamed; partial thickness cartilage
injury which does not bleach the subchondral bone, and injury

– 8 –

of a meniscus, a tendon, a ligament, an intervertebral disk, cardiac muscle in an avascular area or a poor circulation site.

Thus, the present invention provides the following.

1. An implantable synthetic tissue.

2. A synthetic tissue according to item 1, which is biologically organized in the third dimensional direction.

3. A synthetic tissue according to item 1, which has biological integration capability with surroundings.

4. A synthetic tissue according to item 3, wherein the biological integration capability includes capability to adhere to surrounding cells and/or extracellular matrices.

5. A synthetic tissue according to item 1, which comprises cells.

6. A synthetic tissue according to item 1, which is substantially made of cells and a material derived from the cells.

7. A synthetic tissue according to item 1, which is substantially made of cells and an extracellular matrix (ECM) derived from the cells.

8. A synthetic tissue according to item 7, wherein the extracellular matrix contains at least one selected from the group consisting of collagen I, collagen III, vitronectin and fibronectin.

9. A synthetic tissue according to item 7, wherein the
extracellular matrix contains collagen I, collagen III,
vitronectin and fibronectin.

10. A synthetic tissue according to item 7, wherein the
extracellular matrix contains vitronectin.

11. A synthetic tissue according to item 7, wherein the
extracellular matrix contains fibronectin.

12. A synthetic tissue according to item 7, wherein the
extracellular matrix contains collagen I and collagen III,
the collagen constitutes 5% to 25% of the tissue, and the
ratio of the collagen I to the collagen III is between 1:10
and 10:1.

13. A synthetic tissue according to item 7, wherein the
extracellular matrix and the cells are integrated together
into a three-dimensional structure.

14. A synthetic tissue according to item 7, wherein the
extracellular matrix is diffusedly distributed in the tissue.

15. A synthetic tissue according to item 1, wherein an
extracellular matrix is diffusedly distributed, and the
distribution densities of the extracellular matrix in two
arbitrary sections of 1 cm^2 in the tissue have a ratio within
a range of about 1:3 to about 3:1.

16. A synthetic tissue according to item 1, which is
heterologous, allogenic, isologous, or autogenous.

17. A synthetic tissue according to item 1, which is free of scaffolds.

18. A synthetic tissue according to item 1, which is used to implant cells.

19. A synthetic tissue according to item 1, which is large sized.

20. A synthetic tissue according to item 1, which has a volume of at least about 20 mm^3.

21. A synthetic tissue according to item 1, which is flexible.

22. A synthetic tissue according to item 1, which is expandable and contractile.

23. A synthetic tissue according to item 1, which can withstand heart pulsation.

24. A synthetic tissue according to item 1, which is biologically organized in all three dimensional directions.

25. A synthetic tissue according to item 24, wherein the biological integration is selected from the group consisting of internal binding of extracellular matrix, electrical integration, and intercellular signal transduction.

26. A synthetic tissue according to item 1, which has a tissue strength which allows the synthetic tissue to be clinically applicable.

27. A synthetic tissue according to item 26, wherein the strength is a break strength of about 0.02 N to about 2 N.

28. A synthetic tissue according to item 26, wherein the
5   tissue strength is sufficient to provide self-supporting ability.

29. A synthetic tissue according to item 26, wherein the self-supporting ability is characterized in that the
10   synthetic tissue is not substantially broken when the synthetic tissue is picked up using forceps having a tip area of 0.05 to 3.0 mm^2.

30. A synthetic tissue according to item 28, wherein the
15   self-supporting ability is characterized in that the synthetic tissue is not broken when the synthetic tissue is picked up with a hand.

31. A synthetic tissue according to item 26, wherein the
20   site to which the synthetic tissue is intended to be applied, includes a heart.

32. A synthetic tissue according to item 26, wherein the site to which the synthetic tissue is intended to be applied,
25   includes an intervertebral disk, a meniscus, a cartilage, a bone, a ligament, or a tendon.

33. A synthetic tissue according to item 26, wherein:
        the synthetic tissue is a cartilage, an
30   intervertebral disk, a meniscus, a ligament, or a tendon; and

        the synthetic tissue remains attached without an additional fixation procedure, after the synthetic tissue

is implanted into an injured portion of the intra-articular tissue.

34. A method for producing a synthetic tissue, comprising the steps of:

    A) providing cells;

    B) placing the cells in a container, the container having cell culture medium containing an ECM synthesis promoting agent and having a sufficient base area which can accommodate a synthetic tissue having a desired size;

    C) culturing the cells in the container along with the cell culture medium containing the ECM synthesis promoting agent for a period of time sufficient for formation of the synthetic tissue having the desired size; and

    D) detaching the cells from the container.

35. A method according to item 34, wherein a stimulus for inducing tissue contraction is applied in the detaching step.

36. A method according to item 35, wherein the stimulus includes a physical or chemical stimulus.

37. A method according to item 36, wherein the physical stimulus includes shaking of the container, pipetting, or deformation of the container.

38. A method according to item 34, wherein the detaching step includes adding an actin regulatory agent.

39. A method according to item 38, wherein the actin regulatory agent includes a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.

40. A method according to item 39, wherein the actin
depolymerizing agent is selected from the group consisting
of Slingshot, cofilin, cyclase associated protein (CAP),
actin interacting protein 1 (AIP1), actin depolymerizing
factor (ADF), destrin, depactin, actophorin, cytochalasin,
and NGF (nerve growth factor).

41. A method according to item 39, wherein the actin
polymerizing agent is selected from the group consisting
of RhoA, mDi, profilin, Rac1, IRSp53, WAVE2, ROCK, LIM kinase,
cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, lysophosphatidic
acid (LPA), insulin, platelet derived growth factor (PDGF)
a, PDGFb, chemokine, and transforming growth factor (TGF)
β.

42. A method according to item 34, wherein the container
is free of scaffolds.

43. A method according to item 34, wherein the cells are
first cultured in monolayer culture.

44. A method according to item 34, wherein the ECM synthesis
promoting agent includes TGFβ1, TFGβ3, ascorbic acid,
ascorbic acid 2-phosphate, or a derivative or salt thereof.

45. A method according to item 44, wherein the ascorbic acid,
ascorbic acid 2-phosphate, or the derivative or salt thereof
is present at a concentration of at least 0.1 mM.

46. A method according to item 44, wherein the TGFβ1 or TFGβ3
is present at a concentration of at least 1 ng/ml.

47. A method according to item 34, wherein the cells are placed at a concentration of 5×10^4 to 5×10^6 cells per 1 cm^2, and the ECM synthesis promoting agent is ascorbic acid, ascorbic acid 2-phosphate, or a derivative or salt thereof, and the ascorbic acid, ascorbic acid 2-phosphate, or the derivative or salt thereof is provided at a concentration of at least 0.1 mM.

48. A method according to item 34, further comprising causing the synthetic tissue to detach from the container and self-contract.

49. A method according to item 48, wherein the detaching and self-contraction are achieved by providing a physical stimulus to the container.

50. A method according to item 48, wherein the detachment and self-contraction are achieved by providing a chemical stimulus to the container.

51. A method according to item 34, wherein the sufficient period of time is at least 3 days.

52. A method according to item 34, wherein the sufficient period of time is at least 3 days and a period of time required for the synthetic tissue to be spontaneously detached from the container at a maximum.

53. A method according to item 52, wherein the period of time required for the synthetic tissue to be spontaneously detached from the container is at least 40 days.

54. A method according to item 34, further comprising:

causing the synthetic tissue to differentiate.

55. A method according to item 54, wherein the differentiation includes osteogenesis, chondrogenesis, adipogenesis, tendon differentiation, and ligament differentiaion.

56. A method according to item 55, wherein the osteogenesis is performed in medium containing dexamethasone, β-glycerophosphate, and ascorbic acid 2-phosphate.

57. A method according to item 56, wherein the medium contains at least one selected from the group consisting of BMP (bone morphogenetic protein)-2, BMP-4, and BMP-7.

58. A method according to item 55, wherein the chondrogenesis is performed in medium containing pyruvic acid, dexamethasone, ascorbic acid 2-phosphate, insulin, transferrin, and selenious acid.

59. A method according to item 58, wherein the medium contains at least one selected from the group consisting of BMP-2, BMP-4, BMP-7, TGF(transforming frowth factor)-β1 and TGF-β3.

60. A method according to item 54, wherein the differentiation step is performed before or after the detaching step.

61. A method according to item 54, wherein the differentiation step is performed after the detaching step.

62. A method according to item 34, wherein the cell includes

cells of 3 or more passages.

63. A method according to item 34, wherein the cells include cells of 3 to 8 passages.

64. A method according to item 34, wherein the cells are provided at a cell density of 5.0×10^{4} to 5.0×10^{6} cells/cm^2.

65. A method according to item 34, wherein the cells include myoblasts.

66. A method according to item 34, wherein the cells include fat-derived cells.

67. A method according to item 34, wherein the cells include synovium-derived cells.

68. A method according to item 34, wherein the cells include mesenchymal stem cells.

69. A method according to item 68, wherein the mesenchymal stem cells are derived from an adipose tissue, a synovial membrane, a tendon, a bone, or a bone marrow.

70. A method according to item 34, further comprising:
    producing a plurality of the synthetic tissues and attaching the plurality of the synthetic tissues together to be integrated.

71. A cell culture composition for producing a synthetic tissue from cells, comprising:
    A) an element for maintaining the cells; and
    B) an extracellular matrix synthesis promoting

agent.

72. A method according to item 68, wherein the ECM synthesis promoting agent includes TGFβ1, TFGβ3, ascorbic acid, ascorbic acid 2-phosphate, or a derivative or salt thereof.

73. A method according to item 72, wherein TGFβ1 or TFGβ3 is present at a concentration of at least 1 ng/ml, or ascorbic acid, ascorbic acid 2-phosphate, or the derivative or salt thereof is present at a concentration of at least 0.1 mM.

74. A complex for reinforcing a portion of an organism, comprising cells and a component derived from the cells.

75. A complex according to item 74, which has biological integration capability with surroundings.

76. A complex according to item 75, wherein the biological integration capability include capability to adhere to surrounding cells and/or extracellular matrices.

77. A complex according to item 74, which is substantially made of cells and a material derived from the cells.

78. A complex according to item 74, which is substantially made of cells and an extracellular matrix derived from the cells.

79. A synthetic tissue according to item 78, wherein the extracellular matrix is selected from the group consisting of collagen I, collagen III, vitronectin and fibronectin.

80. A complex according to item 78, wherein the extracellular

matrix and the cells are integrated together into a
three-dimensional structure.

81. A complex according to item 78, wherein the extracellular
matrix is provided on a surface of the complex.

82. A complex according to item 78, wherein the extracellular
matrix is diffusedly distributed on a surface of the complex.

83. A complex according to item 74, wherein an extracellular
matrix is diffusedly distributed on a surface of the complex,
and the distribution densities of the extracellular matrix
in two arbitrary sections of 1 cm^2 in the complex have a ratio
within a range of about 1:3 to about 3:1.

84. A complex according to item 78, wherein the extracellular
matrix includes fibronectin or vitronectin.

85. A complex according to item 74, which is heterologous,
allogenic, isologous, or autogenous.

86. A complex according to item 74, wherein the portion
includes a bag-shaped organ.

87. A complex according to item 86, wherein the bag-shaped
organ includes a heart.

88. A complex according to item 74, wherein the portion
includes a bone or cartilage tissue.

89. A complex according to item 74, wherein the portion
includes avascular tissue.

90. A complex according to item 74, wherein the portion includes an intervertebral disk, a meniscus, a ligament, or a tendon.

91. A complex according to item 74, wherein the reinforcement is achieved by replacing the portion with the complex or providing the complex to cover the portion, or both.

92. A complex according to item 74, which resists the expansion and contraction of the portion.

93. A complex according to item 74, which has biological integration.

94. A complex according to item 74, wherein the biological integration selected from the group consisting of internal binding of extracellular matrix, electrical integration, and intercellular signal transduction.

95. A complex according to item 74, which is formed by culturing cells in the presence of an ECM synthesis promoting agent.

96. A complex according to item 74, which has self-supporting ability.

97. A method for reinforcing a portion of an organism, comprising the steps of:

A) replacing the portion with a complex comprising cells and a component derived from the cells or providing the complex to cover the portion, or both; and

B) holding the complex for a sufficient period of time for biologically adhering the complex to the portion.

98. A method according to item 97, wherein the adhesion is achieved by adhesion between extracellular matrix and extracellular matrix.

99. A method according to item 97, which has biological integration capability with surroundings.

100. A method according to item 99, wherein the biological integration capability include capability to adhere to surrounding cells and/or extracellular matrices.

101. A method according to item 97, which is substantially made of cells and a material derived from the cells.

102. A method according to item 97, which is substantially made of cells and an extracellular matrix derived from the cells.

103. A method according to item 102, wherein the extracellular matrix contains one selected from the group consisting of collagen I, collagen III, vitronectin and fibronectin.

104. A method according to item 102, wherein the extracellular matrix contains all of collagen I, collagen III, vitronectin and fibronectin.

105. A method according to item 102, wherein the extracellular matrix contains vitronectin.

106. A method according to item 102, wherein the extracellular matrix contains fibronectin.

107. A method according to item 97, wherein an extracellular matrix is provided on a surface of the complex.

5   108. A method according to item 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex.

109. A method according to item 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex,
10   and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm^2 have a ratio within a range of about 1:3 to about 3:1.

110. A complex according to item 97, wherein an extracellular
15   matrix is diffusedly distributed on a surface of the complex, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm^2 have a ratio within a range of about 1:2 to about 2:1.

20   111. A method according to item 97, which is heterologous, allogenic, isologous, or autogenous.

112. A method according to item 97, wherein the portion includes a bag-shaped organ.

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113. A method according to item 112, wherein the bag-shaped organ includes a heart.

114. A method according to item 97, wherein the complex
30   resists the expansion and contraction of the portion.

115. A method according to item 97, wherein the complex has biological integration.

116. A method according to item 115, wherein the biological integration selected from the group consisting of internal binding of extracellular matrix, electrical integration, and intercellular signal transduction.

117. A method according to item 97, further comprising:
        forming the complex by culturing the cells in the presence of an ECM synthesis promoting agent.

118. A method according to item 97, wherein the portion is a heart and the heart has a disease or disorder selected from the group consisting of heart failure, ischemic heart disease, myocardial infarct, cardiomyopathy, myocarditis, hypertrophic cardiomyopathy, dilated phase hypertrophic cardiomyopathy, and dilated cardiomyopathy.

119. A method according to item 97, wherein the portion includes an avascular lesion.

120. A method according to item 97, wherein the portion includes a vascular lesion.

121. A method according to item 97, wherein the portion includes a bone or a cartilage.

122. A method according to item 97, wherein the portion includes an intervertebral disk, a meniscus, a ligament, or a tendon.

123. A method according to item 97, wherein the portion includes a bone or a cartilage, and the bone or the cartilage is damaged or degenerated.

124. A method according to item 97, wherein the portion includes intractable fracture, osteonecrosis, cartilage injury, meniscus injury, ligament injury, tendon injury, cartilage degeneration, meniscus degeneration, intervertebral disk denaturation, ligament degeneration, or tendon degeneration.

125. A method according to item 97, wherein the sufficient period of time is at least 10 days.

126. A method according to item 97, wherein the complex has self-supporting ability.

127. A method according to item 97, which has Biological integration capability with surroundings.

128. A method according to item 97, which is substantially made of cells and an extracellular matrix derived from the cells.

129. A method according to item 97, further comprising implanting another synthetic tissue.

130. A method according to item 129, wherein the other synthetic tissue is an artificial bone or a microfibrous collagen medical device.

131. A method according to item 97, which is substantially made of cells and an extracellular matrix derived from the cells, wherein the other synthetic tissue is an artificial bone or a microfibrous collagen medical device.

132. A method according to item 130, the artificial bone includes hydroxyapatite.

133. A method for treating a portion of an organism, comprising the steps of:

A) replacing the portion with a complex comprising cells and a component derived from the cells or providing the complex to cover the portion, or both; and

B) holding the complex for a sufficient period of time for restoring a condition of the portion.

134. A method according to item 133, wherein the treatment is for the treatment, prevention, or reinforcement of a disease, disorder, or condition of a heart, a bone, a cartilage, a ligament, a tendon, a meniscus, or an intervertebral disk.

135. A method according to item 133, wherein the complex has self-supporting ability.

136. A method according to item 133, wherein the complex has biological integration capability with surroundings.

137. A method according to item 133, wherein the complex is substantially made of cells and an extracellular matrix derived from the cells.

138. A method according to item 133, further comprising implanting another synthetic tissue in addition to the replacement or coverage of the portion.

139. A method according to item 138, wherein the other synthetic tissue includes an artificial bone or a

microfibrous collagen medical device.

140. A method according to item 133, which is substantially made of cells and an extracellular matrix derived from the cells, wherein the other synthetic tissue includes an artificial bone or a microfibrous collagen medical device.

141. A method according to item 139, the artificial bone includes hydroxyapatite.

142. A method for producing a synthetic tissue, comprising the steps of:

A) providing cells;

B) placing the cells in a container, the container having cell culture medium containing an ECM synthesis promoting agent and having a sufficient base area which can accommodate a synthetic tissue having a desired size;

C) culturing the cells in the container along with the cell culture medium containing the ECM synthesis promoting agent for a period of time sufficient for formation of the synthetic tissue having the desired size; and

D) regulating a thickness of the synthetic tissue by a physical or chemical stimulus to a desired thickness.

143. A method according to item 142, wherein the physical stimulus includes shear stress between the synthetic tissue and the container, deformation of the base of the container, shaking of the container, or pipetting.

144. A method according to item 142, wherein the chemical stimulus is obtained by using a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.

145. A method according to item 144, wherein the actin depolymerizing agent is selected from the group consisting of Slingshot, cofilin, CAP (cyclase associated protein), AIP1 (actin interacting protein 1), ADF (actin depolymerizing factor), destrin, depactin, actophorin, cytochalasin, and NGF (nerve growth factor).

146. A method according to item 144, wherein the actin polymerizing agent is selected from the group consisting of RhoA, mDi, profilin, Rac1, IRSp53, WAVE2, ROCK, LIM kinase, cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, LPA (lysophosphatidic acid), insulin, PDGF (platelet derived growth factor), PDGFb, chemokine, and TGF (transforming growth factor) β.

147. A method according to item 144, wherein the desired thickness is regulated by adjusting a ratio of the actin depolymerizing agent to the actin polymerizing agent.

148. A method according to item 142, further comprising: producing a plurality of the synthetic tissues and attaching the plurality of the synthetic tissues together to be integrated.

149. A tissue complex, comprising an implantable synthetic tissue and another synthetic tissue.

150. A tissue complex according to item 149, wherein the implantable synthetic tissue is substantially made of cells and a material derived from the cells.

151. A tissue complex according to item 149, wherein the

implantable synthetic tissue is substantially made of cells
and an extracellular matrix derived from the cells.

152. A tissue complex according to item 151, wherein the
extracellular matrix is selected from the group consisting
of collagen I, collagen III, vitronectin, and fibronectin.

153. A tissue complex according to item 151, wherein the
extracellular matrix contains all of collagen I, collagen
III, vitronectin, and fibronectin.

154. A tissue complex according to item 149, wherein the
other synthetic tissue includes an artificial bone or a
microfibrous collagen medical device.

155. A tissue complex according to item 154, the artificial
bone includes hydroxyapatite.

156. A tissue complex according to item 149, the implantable
synthetic tissue is biologically integrated with the other
synthetic tissue.

157. A tissue complex according to item 156, wherein the
biological integration is achieved via an extracellular
matrix.

158. A composition for use in producing a synthetic tissue
having a desired thickness, comprising a chemical substance
selected from the group consisting of actin depolymerizing
agents and actin polymerizing agents.

159. A composition according to item 158, wherein the actin
depolymerizing agent is selected from the group consisting

- 28 -

of Slingshot, cofilin, CAP (cyclase associated protein),
AIP1 (actin interacting protein 1), ADF (actin depolymerizing
factor), destrin, depactin, actophorin, cytochalasin, and
NGF (nerve growth factor).

160. A composition according to item 158, wherein the actin
polymerizing agent is selected from the group consisting
of RhoA, mDi, profilin, Racl, IRSp53, WAVE2, ROCK, LIM kinase,
cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, LPA
(lysophosphatidic acid), insulin, PDGF (platelet derived
growth factor) a, PDGFb, chemokine, and TGF (transforming
growth factor) β.

Hereinafter, the present invention will be described
by way of preferable examples. It will be understood by those
skilled in the art that the examples of the present invention
can be appropriately made or carried out based on the
description of the present specification and commonly used
techniques well known in the art. The function and effect
of the present invention can be easily recognized by those
skilled in the art.

The present invention provides a scaffold-free
synthetic tissue or complex. By providing such a
scaffold-free synthetic tissue, a therapeutic method and
a therapeutic agent for providing an excellent therapeutic
result after implantation can be obtained.

The scaffold-free synthetic tissue of the present
invention solves a long outstanding problem with biological
formulations, which is attributed to contamination of the
scaffold itself. Despite the absence of a scaffold, the
therapeutic effect is comparable with, or more satisfactory

- 29 -

than conventional techniques.

In addition, when a scaffold is used, the alignment of implanted cells in the scaffold, the cell-to-cell adhesion, the in vivo alteration of the scaffold itself (eliciting inflammation), the integration of the scaffold to recipient tissue, and the like become problematic. These problems can be solved by the present invention.

The synthetic tissue and the complex of the present invention are also self-organized, and have biological integration inside thereof. Also on this point, the present invention is distinguished from conventional cell therapies.

It is easy to form a three-dimensional structure with the synthetic tissue or complex of the present invention, and thus it is easy to design it into a desired form. The versatility of the synthetic tissue and the complex of the present invention should be noted.

The synthetic tissue and the complex of the present invention have biological integration with recipient tissues, such as adjacent tissues, cells, and the like. Therefore, the post-operational stability is satisfactory, and cells are securely supplied to a local site, for example. An effect of the present invention is that the satisfactory biological integration capability allows the formation of a tissue complex with another synthetic tissue or the like, resulting in a complicated therapy.

Another effect of the present invention is that differentiation can be induced after the synthetic tissue or the complex is provided. Alternatively, differentiation

is induced before providing a synthetic tissue and/or a complex, and thereafter, the synthetic tissue and/or the complex are developed.

Another effect of the present invention is that the implantation of the synthetic tissue of the present invention provides a satisfactory tissue replacement ability and a comprehensive supply of cells for filling or covering an implanted site, compared to conventional cell-only implantation and sheet implantation.

The present invention provides an implantable synthetic tissue with biological integration capability. The above-described features and effects of the present invention make it possible to treat a site which cannot be considered as an implantation site for conventional synthetic products. The synthetic tissue of the present invention has biological integration and actually works in implantation therpies. The synthetic tissue is for the first time provided by the present invention, but is not provided by conventional techniques. The synthetic tissue or composite of the present invention has the sufficient ability to integrating with adjacent tissues, cells or the like during implantation (preferably, due to extracellular matrix). Therefore, post-operational restoration is excellent. Such a synthetic tissue, which has biological integration capability in all of the three dimensions, cannot be achieved by conventional techniques. Therefore, the present invention provides a therapeutic effect which cannot be achieved by conventional synthetic tissue.

In addition, the present invention provides medical treatment which provides a therapeutic effect by filling,

replacing, and/or covering a lesion.

In addition, when the synthetic tissue of the present
invention is used in combination with another synthetic
tissue (e.g., an artificial bone made of hydroxyapatite,
a microfibrous collagen medical device, etc.), the synthetic
tissue of the present invention is biologically integrated
with the other synthetic tissue, so that the acceptance of
the synthetic tissue makes it possible to organize more
complicated tissue complex which is not conventionally
expected.

An extracellular matrix or a cell adhesion molecule,
such as fibronectin, vitronectin, or the like, is distributed
throughout the synthetic tissue of the present invention.
In the cell sheet engineering, a cell adhesion molecule is
localized on a bottom surface of culture cells which is
attached to a Petri dish. In the sheet provided by the cell
sheet engineering, cells are major components of the sheet.
The sheet is intended to provide a mass of cells with an
adhesion molecule attached on the bottom surface. The
synthetic tissue of the present invention is a real "tissue"
such that an extracellular matrix three-dimensionally
integrates with cells. Thus, the present invention is
significantly distinguished from conventional techniques
including the cell sheet engineering.

A cell implanting method without a scaffold has been
reported by a Tokyo Women's Medical University group, in
which a cell sheet is produced using a temperature sensitive
culture dish. Such a cell sheet engineering technique is
internationally appraised due to its originality. However,
a single sheet obtained by this technique is fragile. In

- 32 -

order to obtain the strength that can withstand surgical
manipulation, such as implantation, a plurality of sheets
need to be piled up, for example. Such a problem is solved
by the present invention.

5

        A cell/matrix complex developed by the present
invention does not require a temperature sensitive culture
dish unlike the cell sheet technique. It is easy for the
cell/matrix complex to form into a contractile
10   three-dimensional tissue. There is no technique in the world
other than the present invention, which can produce a
contractile three-dimensional complex having 10 or more
layers of cells without using so-called feeder cells, such
as rodent stroma cells, in about three weeks. By adjusting
15   conditions for matrix synthesis of the cell, it is possible
to produce a complex having a strength which allows surgical
manipulation, such as holding or transferring the complex,
without a special instrument. Therefore, the present
invention is an original, epoch-making technique in the world
20   for reliably and safely perform cell implantation.

                  BRIEF DESCRIPTION OF THE DRAWINGS

        Figure 1 shows macroscopy and histology of exemplary
25   synthetic tissues using synovial cells.

        Figure 2 shows high magnification histology of a
synthetic tissue when ascorbic acid 2-phosphate has a
concentration of 0 mM, 0.1 mM, 1 mM, and 5 mM. As can be
30   seen, Eosin staining of the synthetic tissue is more intense
when ascorbic acid 2-phosphate is added at a concentration
of more than 0.1 mM.

Figure **3** shows a high magnification view of a synthetic tissue on day 3, 7, 14, and 21 of culture. As can be seen, the synthetic tissue is already developed at day 3 but the matrix is scarce. The matrix is getting dense with time.

Figure **4** shows an exemplary stained extracellular matrix in a synthetic tissue derived from synovial cells.

Figure **5** shows exemplary histology of normal tissue (normal skin tissue, synovial membrane tissue, tendon tissue, cartilage tissue, and meniscus tissue).

Figure **6** shows exemplary histology of a commercially available stained collagen sponge as a control. From the left, staining of fibronectin, vitronectin, non-IgG-immune as a negative control and HE staining are shown.

Figure **7** shows the results of collagen content measurement. When 0.1 mM or more of ascorbic acid diphosphate is added, collagene content in the synthetic tissue of the present invention is significantly increased in any of the culture periods. However, substantially no difference among the concentrations of 0.1 mM, 1 mM and 5 mM were found.

Figure **8** shows the results of collagen content measurement. When 0.1 mM or more of ascorbic acid diphosphate is added, collagene content in the synthetic tissue of the present invention is significantly increased in any of the culture periods. However, substantially no difference among the concentrations of 0.1 mM, 1 mM and 5 mM were found.

Figure **9** shows a production of synthetic tissues

using a different number of cells. P represents the number
of passages. Numeral figures in the photograph indicate the
number of cells per cm².

5   Figure **10** shows a production of synthetic tissues
using dishes with different sizes. * indicates culture in
a 35-mm dish. ** indicates culture in a 60-mm dish. ***
indicates culture in a 100-mm dish.

10   Figure **11** shows an exemplary mechanical testing
system for measuring mechanical properties.

          Figure **12** shows a test piece holding portion of an
apparatus for measuring mechanical properties.

15
          Figure **13** shows an enlarged view of an apparatus for
measuring mechanical properties. A test piece is provided
with a marker.

20   Figure **14** shows an enlarged view of a test piece
holding portion.

          Figure **15** shows a disrupted synthetic tissue after
a tensile test.

25
          Figure **16** shows the results (load-deformation
curve) of a tensile test of a synthetic tissue (derived from
synovium) of the present invention.

30   Figure **17** shows the results (stress-strain curve)
of a mechanical properties test of a synthetic tissue (derived
from synovial membrane tissue) of the present invention.

Figure **18** shows an exemplary osteogenic induction experiment of the synthetic tissue of the present invention and the results. The upper half portion shows a scheme for osteogenesis induction. The induction was conducted in the presence of 0.1 μM dexamethasone, 10 mM β-glycerophosphate, and 50 μg/ml ascorbic acid 2-phosphate. The lower left portion shows a control. The middle left portion shows a synthetic tissue differentiated into a bone by osteogenic induction. The middle lane portion shows Alizarin Red staining. The lower right portion shows an ALP-stained control. The middle right portion shows positive ALP-staining in a synthetic tissue by osteogenic induction.

Figure **19** shows the results of chondrogenic differentiation of a synthetic tissue of the present invention. This figure shows cultured synthetic tissues (A) and monolayer (B) using, from the leftmost, normal culture medium, chondrogenic medium, chondrogenic medium plus BPM-2 and chondrogenic medium plus TGF-β1, respectively. Note that A) synthetic tissues have more intense staining of Alcian blue than B) monolayer culture. Also, note that addition of TGF-β results in detachment of a synthetic tissue from the container without mechanical stimulation. (A) Most right lane.

Figure **20** shows semi-quantification of Alcian blue staining for comparison of a synthetic tissue of the present invention with a single cell sheet under chondrogenic stimulation as in Figures **19** and **39**. The left (blue) shows a result of monolayer, and the right (red) shows a result of the synthetic tissue.

Figure **21** shows the expression of various

chondrogenic marker genes (aggrecan, Col II, Sox9, B-actin)
under chondrogenic stimulation.

Figure **22** shows the comparison of the expression of
chondrogenic marker genes within a synthetic tissue and a
monolayer culture of synovial cells under chondrogenic
stimulation as in Figures **19** and **21**.

Figure **23** shows an *in vitro* cartilage implantation
experiment using a synthetic tissue of the present invention
and the results. The upper portion shows a diagram of explant
culture. It is shown that a synthetic tissue is adhered to
a partial thickness cartilage injury (*in vitro*). A
superficial zone was removed, followed by digestion with
chondroitinase ABC (Hinziker EB, JBJS, 1996). The lower left
portion is lower magnification histology (×40). The lower
right portion is higher magnification histology (×200). As
can be seen, the synthetic tissue is tightly attached to
the injured surface.

Figure **24** shows an *in vivo* cartilage implantation
experiment of the present invention and the 10 day results.
A synthetic tissue is firmly adhered to a partial cartilage
injury. The left shows a macroscopic view of the result.
The upper right shows a histology (×40) and the lower right
shows a histology at higher magnification (×200).

Figure **25** shows the adhesion of a synthetic tissue
of the present invention in a cartilage implantation
experiment. The state on day 10 is shown. The left portion
shows the result of HE staining, the middle portion shows
the result of fibronectin staining, and the right portion
shows the result of vitronectin staining.

Figure 26 shows the 1-month result of an in vivo implantation experiment of the present invention. A synthetic tissue is integrated with adjacent cartilage tissue without inflammation. Further, a superficial portion of the synthetic tissue contained a number of fibroblast-like cells (Figure 27), and a deep portion of the synthetic tissue conatined a number of chondrocyte-like cells (Figure 28), indicating the chondrogenesis of the synthetic tissue after the implantation at particularly deep portions.

Figure 27 shows a superficial portion of a synthetic tissue at one month after implantation.

Figure 28 shows a deep portion of a synthetic tissue at one month after implantation.

Figure 29 shows the result of a meniscus repair experiment using a synthetic tissue of the present invention. The left portion of the figure shows that a medial femoral condyle bone and an anterior horn of medial meniscus are exposed. The right figure shows a 6.5-mm defect in a medial knee joint in the anterior horn of medial meniscus.

Figure 30 shows a meniscus repair procedure. The left portion shows a defect before the implantation of a synovial membrane-derived synthetic tissue (lower left). The right portion shows the defect after the implantation of the synovial membrane-derived synthetic tissue.

Figure 31 shows the results of a meniscus repair experiment using a synthetic tissue of the present invention. A visual inspection four weeks after operation is shown.

- 38 -

The upper portion shows a state of a cartilage. It is shown
that substantially no degeneration or injury due to friction
or the like was found on the corresponding chondral surface,
i.e., the meniscal defect was recovered. The lower left and
5   right portions show a repaired defect.

Figure **32** shows the results of a meniscus repair
experiment using a synthetic tissue of the present invention.
The upper portion shows a macroscopic view. The lower left
10 portion shows histology of a repaired tissue. The lower right
portion shows histology of a border between the repaired
tissue and its adjacent meniscus (magnification: x200).

Figure **33** shows an immunohistochemistry of a
15 synthetic tissue derived from adipose tissue. From the left,
H&E staining, fibronectin staining, and vitronectin
staining.

Figure **34** shows the results of osteogenic or chondrogenic
20 induction of a synthetic tissue derived from adipose tissue.

Figure **35** shows the results of a synthetic tissue
25 with osteogenic induction when dexamethasone and
β-glycerophosphate were added in culture medium prior to
a detachment procedure.

Figure **36** shows the results of a synthetic tissue
30 with osteogenic induction when dexamethasone and
β-glycerophosphate were added in culture medium after a
detachment procedure.

Figure **37** shows histology of biological integration of collagen gel containing synovial cells with cartilage after implantation. There is failure in integration observed (arrow).

Figure **38** shows biological integration after implantation to a chondral defect when a synthetic tissue of the present invention was used. The biological integration is completely established.

Figure **39** shows the effect of TGF-β on the detachment of a synthetic tissue. Addition of TGF-β leads to active detachment of the synthetic tissue.

Figure **40** shows a transition in contraction of a synthetic tissue of the present invention where dihydrochytochalasin or Y27632 was added or not. Data is shown in predetermined culture time intervals.

Figure **41** shows a photograph indicating adhesion of a synthetic tissue of the present invention with an artificial bone after fourteen days of culture in chondrogenic medium.

Figure **42** shows histology of a synthetic tissue cultured on a collagen synthetic tissue (CMI collagen sponge, Amgen, USA), which is a microfibrous collagen medical device, for 7 days.

Figure **43** shows a skeletal muscle-derived sheet developed by a synthetic tissue production method without ascorbic acid.

Figure **44** shows a skeletal muscle-derived synthetic

tissue developed by a synthetic tissue production method
with ascorbic acid according to the present invention.

Figure **45** shows histology of the synthetic tissue
as shown in Figure **44** (HE staining).

Figure **46** shows a diagram for explaining a technique
for measuring stress and distortion characteristics to
determine tensile strength.

Figure **47** shows a principle for obtaining a
load/removal of a load curve.

(Description of Sequencing List)

SEQ ID NO.: 1 indicates the nucleic acid sequence
of myosin heavy chain IIa (human: Accession No. NM_017534).

SEQ ID NO.: 2 indicates the amino acid sequence of
myosin heavy chain IIa (human: Accession No. NM_017534).

SEQ ID NO.: 3 indicates the nucleic acid sequence
of myosin heavy chain IIb (human: Accession No. NM_017533).

SEQ ID NO.: 4 indicates the amino acid sequence of
myosin heavy chain IIb (human: Accession No. NM_017533).

SEQ ID NO.: 5 indicates the nucleic acid sequence
of myosin heavy chain IId (IIx) (human: Accession
No. NM_005963).

SEQ ID NO.: 6 indicates the amino acid sequence of
myosin heavy chain IId (IIx) (human: Accession

- 41 -

No. NM_005963).

SEQ ID NO.: 7 indicates the nucleic acid sequence of CD56 (human: Accession No. U63041).

SEQ ID NO.: 8 indicates the amino acid sequence of CD56 (human: Accession No. U63041).

SEQ ID NO.: 9 indicates the nucleic acid sequence of human MyoD (GENBANK Accession No. X56677).

SEQ ID NO.: 10 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 2.

SEQ ID NO.: 11 indicates the nucleic acid sequence of human myogenic factor 5 (MYF5) (GENBANK Accession No. NM_005593).

SEQ ID NO.: 12 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 3.

SEQ ID NO.: 13 indicates the nucleic acid sequence of human myogenin (myogenic factor 4) (GENBANK Accession No. BT007233).

SEQ ID NO.: 14 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 13.

SEQ ID NO.: 15 indicates the nucleic acid sequence of Sox9 (human : Accession No. NM_000346 = a marker specific to a chondrocyte).

SEQ ID NO.: 16 indicates a polypeptide sequence

encoded by the nucleic acid sequence of SEQ ID NO.: 15.

SEQ ID NO.: 17 indicates the nucleic acid sequence of Col 2A1 (human: Accession No. NM_001844 = a marker specific to a chondrocyte).

SEQ ID NO.: 18 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 17.

SEQ ID NO.: 19 indicates the nucleic acid sequence of Aggrecan (human:Accession No. NM_001135 = a marker specific to a chondrocyte).

SEQ ID NO.: 20 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 19.

SEQ ID NO.: 21 indicates the nucei acid sequence of Bone sialoprotein (human: Accession No. NM_004967 = a marker specific to an osteoblast).

SEQ ID NO.: 22 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 21.

SEQ ID NO.: 23 indicates the nucleic acid sequence of Osteocalcin (human: Accession No. NM_199173 = a marker specific to an osteoblast).

SEQ ID NO.: 24 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 23.

SEQ ID NO.: 25 indicates the nucleic acid sequence of GDF5 (human: Accession No. NM_000557 = a marker specific to a ligament cell).

SEQ ID NO.: 26 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 25.

SEQ ID NO.: 27 indicates the nucleic acid sequence of Six1 (human: Accession No. NM_005982 = a marker specific to a ligament cell).

SEQ ID NO.: 28 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 27.

SEQ ID NO.: 29 indicates the nucleic acid sequence of Scleraxis (human: Accession No. BK000280 = a marker specific to a ligament cell).

SEQ ID NO.: 30 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 29.

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention will be described below. It should be understood throughout the present specification that articles for singular forms include the concept of their plurality unless otherwise mentioned. Therefore, articles or adjectives for singular forms (e.g., "a", "an", "the", and the like in English) include the concept of their plurality unless otherwise specified. Also, it should be also understood that terms as used herein have definitions ordinarily used in the art unless otherwise mentioned. Therefore, all technical and scientific terms used herein have the same meanings as commonly understood by those skilled in the relevant art. Otherwise, the present application (including definitions) takes precedence.

- 44 -

(Definition of terms)

The definitions of specific terms used herein are described below.

(Regenerative medicine)

As used herein, the term "regeneration" refers to a phenomenon in which when an individual organism loses a portion of tissue, the remaining tissue grows and recovers. The extent or manner of regeneration varies depending among animal species or among tissues in the same individual. Most human tissues have limited regeneration capability, and therefore, complete regeneration is not expected if a large portion of tissue is lost. In the case of severe damage, a tissue may grow which has strong proliferation capability different from that of lost tissue, resulting in incomplete regeneration where the damaged tissue is incompletely regenerated and the function of the tissue cannot be recovered. In this case, a structure made of a bioabsorbable material is used to prevent a tissue having strong proliferation capability from infiltrating the injured portion of the tissue so as to secure space for proliferation of the damaged tissue. Further, by supplementing with a cell growth factor, the regeneration capability of the damaged tissue is enhanced. Such a regeneration technique is applied to cartilages, bones, hearts, and peripheral nerves, for example. It has been so far believed that cartilages, nerve cells, and cardiac muscles have no or poor regeneration capability. Recently, it was reported that there are tissue (somatic stem cells), which have both the capability of differentiating into these tissues and self-proliferation capability. Expectations are running high for regenerative medicine using stem cells. Embryonic stem cells (ES cells) also have the capability

of differentiating into all tissues. Efforts have been made
to use ES cells for regeneration of complicated organs, such
as kidney, liver, and the like, but have not yet been realized.

5   The term "cell" is herein used in its broadest sense
in the art, referring to a structural unit of tissue of a
multicellular organism, which is capable of self-replicating,
has genetic information and a mechanism for expressing it,
and is surrounded by a membrane structure which isolates
10   the living body from the outside. In the method of the present
invention, any cell can be used as a subject. The number
of cells used in the present invention can be counted through
an optical microscope. When counting using an optical
microscope, the number of nuclei is counted. Tissues are
15   sliced into tissue sections, which are then stained with
hematoxylin-eosin (HE) to variegate nuclei derived from
extracellular matrices (e.g., elastin or collagen) and cells.
.   These tissue sections are observed under an optical
microscope and the number of nuclei in a particular area
20   (e.g., 200 μm × 200 μm) can be estimated to be the number
of cells. Cells used herein may be either
naturally-occurring cells or artificially modified cells
(e.g., fusion cells, genetically modified cells, etc.).
Examples of cell sources include, but are not limited to,
25   a single-cell culture; the embryo, blood of a normally-grown
transgenic animal; a cell mixture of cells derived from
normally-grown cell lines; and the like. Primary culture
cells may be used. Alternatively, subcultrue cells may also
be used. Preferably, when subculture cells are used, the
30   cells are preferably of 3 to 8 passages. As used herein,
cell density may be represented by the number of cells per
unit area (e.g., cm^2).

As used herein, the term "stem cell" refers to a cell
capable of self replication and pluripotency. Typically,
stem cells can regenerate an injured tissue. Stem cells used
herein may be, but are not limited to, embryonic stem (ES)
5   cells or tissue stem cells (also called tissular stem cell,
tissue-specific stem cell, or somatic stem cell). A stem
cell may be an artificially produced cell (e.g., fusion cells,
reprogrammed cells, or the like used herein) as long as it
can have the above-described abilities. Embryonic stem
10 cells are pluripotent stem cells derived from early embryos.
An embryonic stem cell was first established in 1981, and
has been applied to production of knockout mice since 1989.
In 1998, a human embryonic stem cell was established, which
is currently becoming available for regenerative medicine.
15 Tissue stem cells have a relatively limited level of
differentiation unlike embryonic stem cells. Tissue stem
cells are present in tissues and have an undifferentiated
intracellular structure. Tissue stem cells have a higher
nucleus/cytoplasm ratio and have few intracellular
20 organelles. Most tissue stem cells have pluripotency, a long
cell cycle, and proliferative ability beyond the life of
the individual. As used herein, stem cells may be preferably
embryonic stem cells, though tissue stem cells may also be
employed depending on the circumstance.
25

Tissue stem cells are separated into categories of
sites from which the cells are derived, such as the dermal
system, the digestive system, the bone marrow system, the
nervous system, and the like. Tissue stem cells in the dermal
30 system include epidermal stem cells, hair follicle stem cells,
and the like. Tissue stem cells in the digestive system
include pancreatic (common) stem cells, hepatic stem cells,
and the like. Tissue stem cells in the bone marrow system

include hematopoietic stem cells, mesenchymal stem cells, and the like. Tissue stem cells in the nervous system include neural stem cells, retinal stem cells, and the like.

5   As used herein, the term "somatic cell" refers to any cell other than a germ cell, such as an egg, a sperm, or the like, which does not transfer its DNA to the next generation. Typically, somatic cells have limited or no pluripotency. Somatic cells used herein may be
10   naturally-occurring or genetically modified as long as they can achieve the intended treatment.

     The origin of a stem cell is categorized into the ectoderm, endoderm, or mesoderm. Stem cells of ectodermal
15   origin are mostly present in the brain, including neural stem cells. Stem cells of endodermal origin are mostly present in bone marrow, including blood vessel stem cells, hematopoietic stem cells, mesenchymal stem cells, and the like. Stem cells of mesoderm origin are mostly present in
20   organs, including hepatic stem cells, pancreatic stem cells, and the like. As used herein, somatic cells may be derived from any mesenchyme. Preferably, somatic cells derived from mesenchyme may be employed.

25   As cells for use in construction of a synthetic tissue or three-dimensional structure of the present invention, differentiated cells or stem cells derived from the above-described ectoderm, endoderm, or mesoderm may be employed, for example. Examples of such cells include
30   mesenchymal cells. In a certain embodiment, as such cells, myoblasts (e.g., skeletal myoblast, etc.), fibroblasts, synovial cells, and the like may be employed. As such cells, differentiated cells or stem cells can be used as they are.

Cells differentiated from stem cells into a desired direction
can be used.

        As used herein, the term "mesenchymal stem cell"
refers to a stem cell found in mesenchyme.   The term
"mesenchymal stem cell" may be herein abbreviated as "MSC".
Mesenchyme refers to a population of free cells which are
in the asterodal shape or have irregular projections and
bridge gaps between epithelial tissues, and which are
recognized in each stage of development of multicellular
animals.   Mesenchyme also refers to tissue formed with
intracellular   cement   associated   with   the   cells.
Mesenchymal stem cells have proliferation ability and the
ability to differentiate into osteocytes, chondrocytes,
muscle cells, stroma cells, tendon cells, and adipocytes.
Mesenchymal stem cells are employed in order to culture or
grow bone marrow cells or the like collected from patients,
or differentiate them into chondrocytes or osteoblasts.
Mesenchymal stem cells are also employed as reconstruction
material, such as alveolar bones; bones, cartilages or joints
for arthropathy or the like; and the like.   There is a large
demand for mesenchymal stem cells.   A synthetic tissue or
three-dimensional structure of the present invention
comprising mesenchymal stem cells or differentiated
mesenchymal stem cells is particularly useful when a
structure is required in these applications.

        As used herein, the term "isolated" means that
naturally accompanying material is at least reduced, or
preferably substantially completely eliminated, in normal
circumstances.   Therefore, the term "isolated cell" refers
to a cell substantially free of other accompanying substances
(e.g., other cells, proteins, nucleic acids, etc.) in natural

circumstances. The term "isolated tissue" refers to a tissue substantially free of substances other than that tissue (e.g., in the case of synthetic tissues or complexs, substances, scaffolds, sheets, coats, etc. used when the synthetic tissue is produced). As used herein, the term "isolated" refers to a scaffold-free state. Therefore, it will be understood that the synthetic tissue or complex of the present invention in the isolated state may contain components (e.g., medium, etc.) used in the production of it. The term "isolated" in relation to nucleic acids or polypeptides means that, for example, the nucleic acids or the polypeptides are substantially free of cellular substances or culture media when they are produced by recombinant DNA techniques; or precursory chemical substances or other chemical substances when they are chemically synthesized. Isolated nucleic acids are preferably free of sequences naturally flanking the nucleic acid within an organism from which the nucleic acid is derived (i.e., sequences positioned at the 5' terminus and the 3' terminus of the nucleic acid).

As used herein, the term "scaffold-free" indicates that a synthetic tissue does not substantially contain a material (scaffold) which is conventionally used for production of a synthetic tissue. Examples of such a scaffold include, but are not limited to, chemical polymeric compounds, ceramics, or biological formulations such as polysaccharides, collagens, gelatins, hyaluronic acids, and the like. A scaffold is a material which is substantially solid and has a strength which allows it to support cells or tissue.

As used herein, the term "established" in relation to cells refers to a state of a cell in which a particular

- 50 -

property (pluripotency) of the cell is maintained and the cell undergoes stable proliferation under culture conditions. Therefore, established stem cells maintain pluripotency.

5   As used herein, the term "non-embryonic" refers to not being directly derived from early embryos. Therefore, the term "non-embryonic" refers to cells derived from parts of the body other than early embryos. Also, modified embryonic stem cells (e.g., genetically modified or fusion

10   embryonic stem cells, etc.) are encompassed by non-embryonic cells.

        As used herein, the term "differentiated cell" refers to a cell having a specialized function and form (e.g., muscle

15   cells, neurons, etc.). Unlike stem cells, differentiated cells have no or little pluripotency. Examples of differentiated cells include epidermic cells, pancreatic parenchymal cells, pancreatic duct cells, hepatic cells, blood cells, cardiac muscle cells, skeletal muscle cells,

20   osteoblasts, skeletal myoblasts, neurons, vascular endothelial cells, pigment cells, smooth muscle cells, adipocytes, osteocytes, chondrocytes, and the like.

        As used herein, the term "tissue" refers to a group

25   of cells having the same function and form in cellular organisms. In multicellular organisms, constituent cells are usually differentiated so that the cells have specialized functions, resulting in division of labor. Therefore, multicellular organisms are not simple cell aggregations,

30   but constitute organic or social cell groups having a certain function and structure. Examples of tissues include, but are not limited to, integument tissue, connective tissue, muscular tissue, nervous tissue, and the like. Tissue

targeted by the present invention may be derived from any
organ or part of an organism. In a preferable embodiment
of the present invention, tissue targeted by the present
invention includes, but is not limited to, a bones, a cartilage,
5   a tendon, a ligament, a meniscus, an intervertebral disk,
a periosteum, a blood vessel, a blood vessel-like tissue,
a heart, a cardiac valve, a pericardium, a dura mater, and
the like.

10   As used herein, the term "cell sheet" refers to a
structure comprising a monolayer of cells. Such a cell sheet
has at least a two-dimensional biological integration. The
sheet having biological integration is characterized in that
after the sheet is produced, the connection between cells
15   is not substantially destroyed even when the sheet is handled
singly. Such biological integration includes intracellular
connection via an extracellular matrix. It will be
understood that the cell sheet may partially include a two
or three-layer structure.

20

        As used herein, the term "synthetic tissue" refers
to tissue having a state different from natural states.
Typically, a synthetic tissue is herein prepared by cell
culture. Tissue which is removed from an organism and is
25   not subjected to any treatment is not referred to as a synthetic
tissue. Therefore, a synthetic tissue may include materials
derived from organisms and materials not derived from
organisms. The synthetic tissue of the present invention
typically comprises a cell and/or a biological material,
30   and may comprise other materials. More preferably, a
synthetic tissue of the present invention is composed
substantially only of a cell and/or a biological material.
Such a biological material is preferably derived from cells

constituting the tissue (e.g., extracellular matrix, etc.).

As used herein, the term "implantable synthetic tissue" refers to a synthetic tissue, which can be used for
5   actual clinical implantation and can function as a tissue at the implantation site for a certain period of time after implantation. Implantable synthetic tissue typically has sufficient biocompatibility, sufficient affinity, and the like.
10

The sufficient strength of an implantable synthetic tissue varies depending on a part targeted by implantation, but can be determined as appropriate by those skilled in the art.   The strength is sufficient to provide
15   self-supporting ability, and can be determined depending on the environment of implantation. The strength can be measured by measuring stress or distortion characteristics or conducting a creep characteristics indentation test as described below. The strength may also be evaluated by
20   observing the maximum load.

The sufficient size of an implantable synthetic tissue varies depending on a part targeted by implantation, but can be determined as appropriate by those skilled in
25   the art. The size can be determined depending on the environment of implantation.

However, an implantable synthetic tissue preferably has at least a certain size. Such a size (e.g., area) is
30   at least 1 cm^2, preferably at least 2 cm^2, more preferably at least 3 cm^2, even more preferably at least 4 cm^2, at least 5 cm^2, at least 6 cm^2, at least 7 cm^2, at least 8 cm^2, at least 9 cm^2, at least 10 cm^2, at least 15 cm^2, or at least 20 cm^2.

An essence of the present invention is that a synthetic tissue
of any size (area, volume) can be produced, i.e., the size
is not particularly limited.

5   When the size is represented by the volume, the size
may be, but is not limited to, at least 2 mm^3, or at least
40 mm^3. The size may be 2 mm^3 or less or 40 mm^3 or more.

The sufficient thickness of an implantable synthetic
10   tissue varies depending on a part targeted by implantation,
but can be determined as appropriate by those skilled in
the art. The thickness can be determined depending on the
environment of implantation. The thickness may exceed 5 mm.
When an implantable synthetic tissue is implanted into the
15   heart, the tissue may only have these minimum thicknesses.
When implantable synthetic tissue is used in other
applications, the tissue may preferably have a greater
       thickness. In such a case, for example, an implantable
synthetic tissue has preferably a thickness of at least 2 mm,
20   more preferably at least 3 mm, and even more preferably 5 mm.
For example, when an implantable synthetic tissue is applied
to a bone, a cartilage, a ligament, a tendon, or the like,
similar to the case of a heart, the tissue has a thickness
of at least about 1 mm (e.g., at least 2 mm, more preferably
25   at least 3 mm, and even more preferably 5 mm), or 5 mm or
more or less than 1 mm. An essence of the present invention
is that a synthetic tissue or complex of any thickness can
be produced, i.e., the size is not particularly limited.

30   The sufficient biocompatibility of implantable
synthetic tissue varies depending on a part targeted by
implantation, but can be determined as appropriate by those
skilled in the art. However, an implantable synthetic tissue

- 54 -

preferably has at least a certain level of biocompatibility.
Typically, a desired level of biocompatibility is, for
example, such that biological integration to surrounding
tissues is achieved without any inflammation, any immune
5   reaction or the like. The present invention is not limited
to this. In some cases (e.g., corneas, etc.), an immune
reaction is less likely to occur. Therefore, an implantable
synthetic tissue has biocompatibility to an extent, which
achieves the object of the present invention even when an
10   immune reaction is likely to occur in other organs. Examples
of parameters indicating biocompatibility include, but are
not limited to, the presence or absence of an extracellular
matrix, the presence or absence of an immune reaction, the
degree of inflammation, and the like. Such biocompatibility
15   can be determined by examining the compatibility of a
synthetic tissue at an implantation site after implantation
(e.g., confirming that an implanted synthetic tissue is not
destroyed). See "Hito Ishoku Zoki Kyozetsu Hanno no Byori
Soshiki Shindan Kijyun Kanbetsu Shindan to Seiken Hyohon
20   no Toriatsukai (Zufu) Jinzo Ishoku, Kanzo Ishoku Oyobi Shinzo
Ishoku [Pathological Tissue Diagnosis Criterion for Human
Transplanted Organ Rejection Reaction   Handling   of
Differential Diagnosis and Biopsy Specimen (Illustrated
Book) Kidney Transplantation, Liver Transplantation and
25   Heart   Transplantation]"   The   Japan   Society   for
Transplantation and The Japanese Society for Pathology
editors, Kanehara Shuppan Kabushiki Kaisha (1958).
According to this document, biocompatibility is divided into
Grade 0, 1A, 1B, 2, 3A, 3B, and 4. At Grade 0 (no acute
30   rejection), no acute rejection reaction, cardiomyocyte
failure, or the like is found in biopsy specimens. At Grade
1A (focal, mild acute rejection), there is focal infiltration
of   large   lymphocytes   around   blood   vessels   or   into

interstitial tissue, while there is no damage to
cardiomyocytes. This observation is obtained in one or a
plurality of biopsy specimens. At Grade 1B (diffuse, mild
acute rejection), there is diffuse infiltration of large
lymphocytes around blood vessels or into interstitial tissue
or both, while there is no damage to cardiomyocytes. At Grade
2 (focal, moderate acute rejection), there is a single
observed infiltration focus of inflammatory cells clearly
bordered from the surrounding portions. Inflammation cells
are large activated lymphocytes and may include eosinophils.
Damage to cardiomyocytes associated with modification of
cardiac muscle is observed in lesions. At Grade 3A
(multifocal, moderate acute rejection), there are multiple
infiltration foci of inflammatory cells which are large
activated lymphocytes and may include eosinophils. Two or
more of the multiple inflammatory infiltration foci of
inflammatory cells have damages to cardiomyocytes. In some
cases, there is also rough infiltration of inflammatory cells
into the endocardium. The infiltration foci are observed
in one or a plurality of biopsy specimens. At Grade 3B
(multifocal, borderline severe acute rejection), there are
more confluent and diffuse infiltration foci of inflammatory
cells found in more biopsy specimens than those observed
at Grade 3A. There is infiltration of inflammatory cells
including large lymphocytes and eosinophils, in some cases
neutrophils, as well as damage to cardiomyocytes. There is
no hemorrhage. At Grade 4 (severe acute rejection), there
is infiltration of various inflammatory cells including
activated lymphocytes, eosinophils, and neutrophils. There
is always damage to cardiomyocytes and necrosis of
cardiomyocytes. Edema, hemorrhage, and/or angitis are also
typically observed. Infiltration of inflammatory cells
into the endocardium, which is different from the "Quilty"

effect, is typically observed. When a therapy is strongly
conducted using an immunosuppressant for a considerably long
period of time, edema and hemorrhage may be more significant
than infiltration.

5

The sufficient affinity of an implantable synthetic
tissue varies depending on a part targeted by implantation,
but can be determined as appropriate by those skilled in
the art. Examples of parameters for affinity include, but
10   are not limited to, biological integration capability between
an implanted synthetic tissue and its implantation site,
and the like. Such affinity can be determined based on the
presence of biological integration at an implantation site
after implantation. Preferable affinity is herein such that
15   an implanted synthetic tissue has the same function as that
of a site in which the tissue is implanted, for example.

As used herein, the term "self-supporting ability"
in relation to a tissue (e.g., a synthetic tissue, etc.)
20   refers to a property of the synthetic tissue such that when
it is restrained on at least one point thereof, it is not
substantially destroyed. Self-supporting ability is herein
observed if a tissue (e.g., a synthetic tissue) is picked
up by using forceps with a tip having a thickness of 0.5
25   to 3.0 mm (preferably, forceps with a tip having a thickness
of 1 to 2 mm or 1 mm; the forceps preferably have a bent
tip) and the tissue is not substantially destroyed. Such
forceps are commercially available (e.g., from Natsume
Seisakusho, etc.). A force exerted for picking up a tissue
30   is comparable with a force typically exerted by a medical
practioner   handing   a   tissue.   Therefore,   the
self-supporting ability of a tissue can also be represented
by a property such that the tissue is not destroyed when

it is picked up by a hand. Such forceps are, for example,
but are not limited to, a pair of curved fine forceps (e.g.,
No. A-11 (tip: 1.0 mm in thickness) and No. A-12-2 (tip:
0.5 mm in thickness) commercially available from Natsume
Seisakusho). A bent tip is suitable for picking up a
synthetic tissue. The forceps are not limited to a bent tip
type.

When a joint is treated, replacement is majorly
performed. The strength of a synthetic tissue of the present
invention required in such a case is such that a minimum
self-supporting ability is obtained. Cells contained in the
synthetic tissue are subsequently replaced with cells in
an affected portion. The replacing cells produce a matrix
which enhances the mechanical strength, so that the joint
is healed. It will also be understood that the present
invention may be used in conjunction with an artificial joint.

In the present invention, self-supporting ability
plays an important role in evaluating the supporting ability
of a synthetic tissue which is actually produced. When a
synthetic tissue of the present invention is produced, the
synthetic tissue is formed in the shape of a cell sheet in
a container. Thereafter, the sheet is detached. With
conventional techniques, the sheet is usually destroyed due
to lack of self-supporting ability. Therefore, in
conventional technology, an implantable synthetic tissue
cannot be substantially produced. Especially, when a
large-sized synthetic tissue is required, conventional
techniques are not adequate. According to the technique of
the present invention, a synthetic tissue can be produced,
which has a sufficient strength which allows the tissue to
be detached from a container without being destroying, i.e.,

the tissue already has self-supporting ability when being
detached. This is true even when the synthetic tissue is
in the form of a monolayer sheet before being detached. It
will be understood that the monolayer may partially include
5   a two or three-layer structure. Thus, it will be understood
that the synthetic tissue of the present invention is
applicable in substantially any chosen therapy. In
addition, typically, after a synthetic tissue is produced
and detached, the strength and self-supporting ability of
10 the synthetic tissue are increased as observed in the present
invention. Therefore, in the present invention, it will be
understood that the self-supporting ability evaluated upon
production may be an important aspect. In the present
invention, the strength upon implantation is also important.
15 It may also be important to evaluate the self-supporting
ability of a synthetic tissue when a predetermined time has
passed after the production of the tissue. Therefore, it
will be understood that the self supporting ability at the
time of implantation after transport, can be determined by
20 calculating the time that has elapsed since production of
the tissue, based on the above-described relationship.

        As used herein, the term "membranous tissue" refers
to a tissue in the form of membrane and is also referred
25 to as "planar tissue". Examples of membranous tissue include
tissues of organs (e.g., periosteum, pericardium, dura mater,
cornea, etc.).

        As used herein, the term "organ" refers to a structure
30 which is a specific part of an individual organism where
a certain function of the individual organism is locally
performed and which is morphologically independent.
Generally, in multicellular organisms (e.g., animals and

plants), organs are made of several tissues in specific
spatial arrangement and tissue is made of a number of cells.
Examples of such organs include, but are not limited to,
skin, blood vessel, cornea, kidney, heart, liver, umbilical
5   cord, intestine, nerve, lung, placenta, pancreas, brain,
joint, bone, cartilage, peripheral limbs, retina, and the
like. Examples of such organs include, but are not limited
to, organs of the skin system, the parenchyma pancreas system,
the pancreatic duct system, the hepatic system, the blood
10   system, the myocardial system, the skeletal muscle system,
the osteoblast system, the skeletal myoblast system, the
nervous system, the blood vessel endothelial system, the
pigment system, the smooth muscle system, the fat system,
the bone system, the cartilage system, and the like.
15
        As used herein, the term "bag-shaped organ" refers
to an organ which has a three-dimensional expanse and the
inside of which may be connected via a tubular tissue to
the outside. Examples of bag-shaped organs include, but are
20   not limited to, heart, liver, kidney, stomach, spleen, and
the like.

        In one embodiment, the present invention targets an
intervertebral disk, a cartilage, a joint, a bone, a meniscus,
25   a synovial membrane, a ligament, a tendon, and the like.
In a preferable embodiment, the present invention targets
blood vessels, blood vessel-like tissue, heart, heart valves,
pericardia, dura mater, cornea, and bones. In another
preferable embodiment, examples of organs targeted by the
30   present invention include, but are not limited to, skeletal
muscle, fat, and the like in addition to what is described
above.

As used herein, the term "cover" or "wrap" in relation
to a synthetic tissue, a three-dimensional structure, or
the like, which is wrapped around a certain part (e.g., an
injured site, etc.), means that the synthetic tissue or the
5   like is arranged so as to cover the part (i.e., conceal an
injury or the like). The terms "wrap" and "arrange (or
locate) so as to cover" are used interchangeably. By
observing the spatial relationship between the part and the
synthetic tissue or the like, it can be determined whether
10   or not the part is covered by the synthetic tissue or the
like. In a preferable embodiment, in a covering step, a
synthetic tissue or the like can be wrapped one turn around
a certain site.

15   As used herein, the term "replace" means that a lesion
(a site of an organism) is replaced, and cells which have
originally been in a lesion are replaced with cells supplied
by a synthetic tissue or a complex according to the present
invention. Examples of a disease for which replacement is
20   suitable include, but not limited to, a ruptured site, and
the like. The term "fill" may be used in place of the term
"replace" in the present specification.

A "sufficient time required for a synthetic tissue
25   to biologically integrate with a part" herein varies
depending on a combination of the part and the synthetic
tissue, but can be determined as appropriate by those skilled
in the art based on the combination. Examples of such a time
include, but are not limited to, 1 week, 2 weeks, 1 month,
30   2 months, 3 months, 6 months, 1 year, and the like, after
operation. In the present invention, a synthetic tissue
preferably comprises substantially only cells and materials
derived from the cells, and therefore, there is no particular

material which needs to be extracted after operation.
Therefore, the lower limit of the sufficient time is not
particularly important. Thus, in this case, a longer time
is more preferable. If the time is substantially extremely
long, reinforcement is substantially completed.

As used herein, the term "immune reaction" refers
to a reaction due to the dysfunction of immunological
tolerance between a graft and a host. Examples of immune
reactions include, but are not limited to, a hyperacute
rejection reaction (within several minutes after
implantation) (immune reaction caused by antibodies, such
as β-Gal or the like), an acute rejection reaction (reaction
caused by cellular immunity about 7 to 21 days after
implantation), a chronic rejection reaction (rejection
reaction caused by cellular immunity 3 or more months after
operation), and the like.

As used herein, the elicitation of an immune reaction
can be confirmed by pathological and histological examination
of the type, number, or the like of infiltration of
(immunological) cells into implanted tissue using staining
(e.g., HE staining, etc.), immunological staining, or
microscopic inspection of tissue sections.

As used herein, the term "calcification" refers to
precipitation of calcareous substances in organisms.

"Calcification" in vivo can be determined herein by
staining (e.g., Alizarin Red staining) and measuring calcium
concentration. Specifically, implanted tissue is taken
out; the tissue section is dissolved by acid treatment or
the like; and the atomic absorption of the solution is measured

- 62 -

by a trace element quantifying device.

As used herein, the term "within organism(s) (or in organism(s))" or "*in vivo*" refers to the inner part of organism(s). In a specific context, "within organism(s)" refers to a position at which a subject tissue or organ is placed.

As used herein, "*in vitro*" indicates that a part of an organism is extracted or released outside the organism for various purposes of research (e.g., in a test tube). The term *in vitro* is in contrast to the term *in vivo*.

As used herein, the term "ex vivo" refers to a series of operations where target cells into which a gene will be introduced are extracted from a subject; a therapeutic gene is introduced *in vitro* into the cells; and the cells are returned into the same subject.

As used herein, the term "material derived from cell(s)" refers to any material originating from the cell(s), including, but not being limited to, materials constituting the cell(s), materials secreted by the cell(s), materials metabolized by the cell(s), and the like. Representative examples of materials derived from cells include, but are not limited to, extracellular matrices, hormones, cytokines, and the like. Materials derived from cells typically have substantially no adverse effect on the cells and their hosts. Therefore, when the material is contained in a synthetic tissue, a three-dimensional structure, or the like, the material typically has substantially no adverse effect on the synthetic tissue, three-dimensional structure, or the like.

- 63 -

As used herein, the term "extracellular matrix" (ECM)
refers to a substance existing between somatic cells no matter
whether the cells are epithelial cells or non-epithelial
5   cells. Extracellular matrices are typically produced by
cells, and therefore, are biological materials.
Extracellular matrices are involved in supporting tissue
as well as in internal environmental structure essential
for survival of all somatic cells. Extracellular matrices
10   are generally produced from connective tissue cells. Some
extracellular matrices are secreted from cells possessing
basal membrane, such as epithelial cells or endothelial cells.
Extracellular matrices are roughly divided into fibrous
components and matrices filling there between. Fibrous
15   components include collagen fibers and elastic fibers. A
basic component of matrices is a glycosaminoglycan (acidic
mucopolysaccharide), most of which is bound to
non-collagenous protein to form a polymer of a proteoglycan
(acidic mucopolysaccharide-protein complex). In addition,
20   matrices include glycoproteins, such as laminin of basal
membrane, microfibrils around elastic fibers, fibers,
fibronectins on cell surfaces, and the like. Particularly
differentiated tissue has the same basic structure. For
example, in hyaline cartilage, chondroblasts
25   characteristically produce a large amount of cartilage
matrices including proteoglycans. In bones, osteoblasts
produce bone matrices which cause calcification. Herein,
examples of typical extracellular matrix include, but not
limited to, collagen I, collagen III, collagen V, elastin,
30   vitronectin, fibronectin, proteoglycans (for example,
decolin, byglican, fibromodulin, lumican, hyaluronic acid,
etc.). Various types of extracellular matrix may be utilized
in the present invention as long as cell adhesion is achieved.

In one embodiment of the present invention, the
synthetic tissue, three-dimensional structure, or the like
of the present invention may be advantageously similar to
the composition of an extracellular matrix (e.g., elastin,
collagen (e.g., Type I, Type III, Type IV, etc.), laminin,
etc.) of a site of an organ for which implantation is intended.
In the present invention, extracellular matrices include
cell adhesion molecules. As used herein, the terms "cell
adhesion molecule" and "adhesion molecule" are used
interchangeably, referring to a molecule capable of mediating
the joining of two or more cells (cell adhesion) or adhesion
between a substrate and a cell. In general, cell adhesion
molecules are divided into two groups: molecules involved
in cell-cell adhesion (intercellular adhesion) (cell-cell
adhesion molecules) and molecules involved in
cell-extracellular matrix adhesion (cell-substrate
adhesion) (cell-substrate adhesion molecules). A synthetic
tissue or three-dimensional structure of the present
invention typically comprises such a cell adhesion molecule.
Therefore, cell adhesion molecules herein include a protein
of a substrate and a protein of a cell (e.g., integrin, etc.)
in cell-substrate adhesion. A molecule other than proteins
falls within the concept of cell adhesion molecule as long
as it can mediate cell adhesion.

It should be noted that the synthetic tissue or
complex of the present invention comprises cells and a ▉
material (natively) derived from the cell. ▉ Therefore, such
materials including ECMs form a complicated composition
containg collagen I, collagen III, collagen V, elastin,
fibronectin, vitronectin, proteoglycans (for example,
decolin, byglican, fibromodulin, lumican, hyaluronic acid,

etc.). Conventionally a synthetic tissue containg such
cell-derived ingredients has not been provided. To obtain
a synthetic tissue having such a composition is substantially
impossible when an artificial material is used. Thus, a
composition containing such ingredients (particularly ,
collagen I, collagen III and the like) is recognized to be
a native composition.

More preferably, an extracellular matrix includes
all the collagen (for example, Types I, Type III, etc.),
vitronectin, and fibronectin. Especially, a synthetic
tissue containing vitronectin and/or fibronectin has not
been provided before. Therefore, the synthetic tissue and
the complex according to the present invention are recognized
to be new in this regard.

As used herein, the term "provided" or "distributed"
in relation to an extracellular matrix and the synthetic
tissue of the present invention indicates that the
extracellular matrix is present in the synthetic tissue.
It should be understood that such superficial provision can
be visualized and observed by immunologically staining an
extracellular matrix of interest.

As used herein, the term "in a diffused manner" or
"diffusedly" in relation to the distribution of an
extracellular matrix indicates that the extracellular matrix
is not localized. Such distribution of an extracellular
matrix has a ratio of the distribution densities of two
arbitrary sections of 1 cm^2 within a range of typically about
1:10 to about 10:1, and representatively about 1:3 to about
3:1, and preferably about 1:2 to about 2:1, and more preferably
about 1:1 (i.e., substantially evenly distributed over the

synthetic tissue.   When an extracellular matrix is
distributed on a surface of the synthetic tissue of the present
invention, but not localized, the synthetic tissue of the
present invention has biological integration capability

5   evenly with respect to the surrounding. Therefore, the
synthetic tissue of the present invention has an excellent
effect of recovery after implantation.

For cell-cell adhesion, cadherin, a number of
10   molecules belonging in an immunoglobulin superfamily (NCAML1,
ICAM, fasciclin II, III, etc.), selectin, and the like are
known, each of which is known to join cell membranes via
a specific molecular reaction. Therefore, in one embodiment,
the synthetic tissue, three-dimensional structure, or the

15   like of the present invention preferably has substantially
the same composition of cadherin, immunoglobulin superfamily
molecules, or the like as that of a site for which implantation
is intended.

20   Thus, various molecules are involved in cell adhesion
and have different functions. Those skilled in the art can
appropriately select a molecule to be contained in a synthetic
tissue or three-dimensional structure of the present
invention depending on the purpose. Techniques for cell

25   adhesion are well known as described above and as described
in, for example, "Saibogaimatorikkusu -Rinsho heno Oyo-
[Extracellular matrix -Clinical Applications-], Medical
Review.

30   It can be determined whether or not a certain molecule
is a cell adhesion molecule, by an assay, such as biochemical
quantification (an SDS-PAG method, a labeled-collagen method,
etc.), immunological quantification (an enzyme antibody

method, a fluorescent antibody method, an immunohistological
study, etc.), a PCR method, a hybridization method, or the
like, in which a positive reaction is detected. Examples
of such a cell adhesion molecule include, but are not limited
5   to, collagen, integrin, fibronectin, laminin, vitronectin,
fibrinogen, an immunoglobulin superfamily member (e.g., CD2,
CD4, CD8, ICM1, ICAM2, VCAM1), selectin, cadherin, and the
like. Most of these cell adhesion molecules transmit into
a cell an auxiliary signal for cell activation due to
10   intercellular interaction as well as cell adhesion.
Therefore, an adhesion molecule for use in an implant of
the present invention preferably transmits an auxiliary
signal for cell activation into a cell. This is because cell
activation can promote growth of cells originally present
15   or aggregating in a tissue or organ at an injured site after
application of an implant thereto. It can be determined
whether or not such an auxiliary signal can be transmitted
·   into a cell, by an assay, such as biochemical quantification
(an SDS-PAG method, a labeled-collagen method, etc.),
20   immunological quantification (an enzyme antibody method,
a fluorescent antibody method, an immunohistological study,
etc.), a PDR method, a hybridization method, or the like,
in which a positive reaction is detected.

25   An example of a cell adhesion molecule is cadherin
which is present in many cells capable of being fixed to
tissue. Cadherin can be used in a preferable embodiment of
the present invention. Examples of a cell adhesion molecule
in cells of blood and the immune system which are not fixed
30   to tissue, include, but are not limited to, immunoglobulin
superfamily molecules (LFA-3, CD2, CD4, CD8, ICAM-1, ICAM2,
VCAM1, etc.); integrin family molecules (LFA-1, Mac-1,
gpIIbIIIa, p150, p95, VLA1, VLA2, VLA3, VLA4, VLA5, VLA6,

- 68 -

etc.); selectin family molecules (L-selectin, E-selectin, P-selectin, etc.), and the like. Therefore, such a molecule may be useful for treatment of a tissue or organ of blood and the immune system.

Nonfixed cells need to be adhered to a specific tissue in order to act on the tissue. In this case, it is believed that cell-cell adhesion is gradually enhanced via a first adhesion by a selectin molecule or the like which is constantly expressed and a second adhesion by a subsequently activated integrin molecule. Therefore, in the present invention, a cell adhesion molecule for mediating the first adhesion and another cell adhesion molecule for mediating the second adhesion may be used together.

As used herein, the term "actin regulatory agent" refers to a substance which interacts directly or indirectly with actin in cells to change the form or state of the actin. It should be understood that actin regulatory agents are categorized into two classes, actin depolymerizing agents and actin polymerizing agents, depending on the action on actin. Examples of actin depolymerizing agents include, but are not limited to, Slingshot, cofilin, CAP (cyclase associated protein), ADF (actin depolymerizing factor), destrin, depactin, actophorin, cytochalasin, NGF (nerve growth factor), and the like. Examples of actin polymerizing agents include, but are not limited to, RhoA, mDi, profilin, Rac1, IRSp 53, Wave2, profilin, ROCK, Lim kinase, cofilin, cdc42, N-WASP, Arp2/3, Drf3, IRSp53, Mena, LPA (lysophosphatidic acid), insulin, PDGF (platelet-derived growth factor) a, PDGFb, chemokine, TGF (transforming growth factor) b, and the like. The above-described actin regulatory agents include some substances which can be

- 69 -

identified by the following assay. Interaction of an actin
regulatory agent with respect to actin is assayed as follows.
Actin is visualized using an actin staining reagent
(Molecular Probes, Texas Red-X phalloidin) or the like. By
5   observing actin aggregation or cell outgrowth under a
microscope, the presence of the interaction is determined
by confirming the aggregation and reconstruction of actin
and/or an increase in the cell outgrowth rate. The
determination may be performed quantitatively or
10   qualitatively. The above-described actin regulatory agents
are used in the present invention so as to promote the
detachment or a multilayer structure of the synthetic tissue.
When an actin regulatory agent used in the present invention
is derived from an organism, the organism may be a mammalian
15   species, such as human, mouse, bovine, or the like.

The above-described agents involved in actin
polymerization control actin polymerization in relation to
Rho and the examples of the agents include the follwing (see,
20   for example, "Saibokokkaku/Undo ga wakaru (Understanding
of cytoskeleton/movement)", (Ed./Hiroaki Miki), Yodo-sha).

Actin polymerization (see Takenaka T et al. J.Cell
Sci., 114: 1801-1809, 2001)

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RhoA → mDi → profilin ⇒ actin polymerization

RhoA → ROCK/Rho → LIM kinase → phosphorylation of
(suppression) ⇒ actin polymerization

30

Rac1 → IRSp53 → WAVE2 → profilin, Arp2/3 ⇒ actin
polymerization

cdc42 → N-WASP → profilin, Arp2/3 ⇒ actin
polymerization

cdc42 → Drf3 → IRSp53 → Mena ⇒ actin polymerization

(In the above descriptions, → indicates a singal
transduction pathway such as phosphorylation. In the
present invention any agent involved in such a pathway can
be utilized.

Actin depolymerization

Slingshot   →   dephosphorization   of   cofilin
(activation) ⇒ actin depolymerization

Actin depolymerization is controlled by a balance
between phosphorylation by LIM kinase activity of cofilin
and dephosphorization by Slingshot. As another agent for
activating cofilin, CAP(cyclase-associated protein) and
AIP1(actin-interacting-protein 1) are identified. It is
recognized that any suitable agent can be used.

LPA (lysophosphatidic acid) of any chain length can
be used.

Any chemokine can be used. However, examples of
prefereable chemokine include interleukin 8, MIP-1, SDF-1
and the like.

Any TGFβ can be used. However, examples of
preferable TGFβ include TGF-β1 and TGF-β3. TGF-β1 and TGF-β3
has an extracellular matrix generation promoting activity.
Thus, in the present invention, TGF-β1 and TGF-β3 are used

with an attention.

As used herein, the term "tissue strength" refers
to a parameter which indicates a function of a tissue or
organ and a physical strength of the tissue or organ. Tissue
strength can be generally determined by measuring tensile
strength (e.g., break strength, modulus of rigidity, Young's
modulus, etc.). Such a general tensile test is well known.
By analyzing data obtained by a general tensile test, various
data, such as break strength, modulus of rigidity, Young's
modulus, and the like, can be obtained. These values can
be herein used as indicators of tissue strength. Typically,
tissue strength which allows clinical applications is herein
required.

The tensile strength of a synthetic tissue,
three-dimensional structure, or the like of the present
invention can be determined by measuring the stress and
distortion characteristics thereof. Briefly, a load is
applied to a sample; the resultant distortion and the load
are input to respective A/D converters (e.g., ELK-5000)
(1 ch: distortion, 2 ch: load); the stress and distortion
characteristics are measured to determine the tensile
strength of the sample (Figure 46). Tensile strength can
also be determined by testing creep characteristics. A creep
characteristics indentation test is conducted to investigate
how a sample is extended over time while a constant load
is applied to the sample. For small materials, thin materials,
and the like, an indentation test is conducted using, for
example, a triangular pyramid-shaped indenter with a tip
having a radius of about 0.1 μm to about 1 μm. Initially,
the indenter is pushed into a test piece so that a load is
given to the test piece. When the indenter reaches from

several tens of nanometers to several micrometers deep in
the test piece, the indenter is drawn off to remove the load.
Figure 47 shows a load/removal of load curve obtained by
the above-described test method. Rigidity, Young's modulus,
or the like can be obtained based on the behavior of the
load and the push depth derived from the curve.

The tensile strength of the synthetic tissue of the
present invention may be low. The tensile strength becomes
higher when the matrix concentration is increased, and
becomes lower when the cell to matrix ratio is increased.
The present invention is characterized in that the strength
can be adjusted as necessary. The present invention is also
characterized in that the strength can be high or low relative
to that of a tissue to be implanted. Therefore, it is
recognized that the strength can be set to comply with any
desired site.

As used herein, the term "physiologically active
substance" refers to a substance capable of acting on a cell
or tissue. Physiologically active substances include
cytokines and growth factors. A cellular physiologically
active substance may be naturally-occurring or synthesized.
Preferably, a cellular physiologically active substance is
one that is produced by a cell or one that has a function
similar thereto. As used herein, a cellular physiologically
active substance may be in the form of a protein or a nucleic
acid or in other forms. In actual practice, cellular
physiologically active substances are typically proteins.
In the present invention, a physiologically active substance
may be used to promote the affinity of an implanted synthetic
tissue of the present invention, for example.

        The term "cytokine" is used herein in the broadest
sense in the art and refers to a physiologically active
substance which is produced from a cell and acts on the same
or different cell. Cytokines are generally proteins or
5 polypeptides having a function of controlling an immune
response, regulating the endocrine system, regulating the
nervous system, acting against a tumor, acting against a
virus, regulating cell growth, regulating cell
differentiation, or the like. Cytokines are herein in the
10 form of a protein or a nucleic acid or in other forms. In
actual practice, cytokines are typically proteins.

        The terms "growth factor" or "cell growth factor"
are used herein interchangeably and each refers to a substance
15 which promotes or controls cell growth. Growth factors are
also called "proliferation factors" or "development factors".
Growth factors may be added to cell or tissue culture medium,
substituting for serum macromolecules. It has been revealed
that a number of growth factors have a function of controlling
20 differentiation in addition to a function of promoting cell
growth.

        Examples of cytokines representatively include, but
are not limited to, interleukins, chemokines, hematopoietic
25 factors such as colony stimulating factors, a tumor necrosis
factor, interferons, a platelet-derived growth factor (PDGF),
an epidermal growth factor (EGF), a fibroblast growth factor
(FGF), a hepatocyte growth factor (HGF), a vascular
endothelial cell growth factor (VEGF), cardiotrophin, and
30 the like, which have proliferative activity.

        Cellular physiologically active substances, such as
cytokines, growth factors, and the like, typically have

redundancy in function. Accordingly, reference herein to
a particular cytokine or growth factor by one name or function
also includes any other names or functions by which the factor
is known to those of skill in the art, as long as the factor
5   has the activity of a cellular physiologically active
substance for use in the present invention. Cytokines or
growth factors can be used in a therapeutic or pharmaceutical
agent according to a preferable embodiment of the present
invention as long as they have preferable activity as
10   described herein.

        Therefore, in one embodiment of the present invention,
it was revealed that when such a cytokine or growth factor
(e.g., BMP-2, etc.) is provided to an implantation site (e.g.,
15   an injured site of a cartilage, etc.) concomitantly with
a synthetic tissue or three-dimensional structure of the
present invention, the affinity of the synthetic tissue or
three-dimensional structure and an improvement in the
function of the implantation site are observed. Thus, the
20   present invention also provides such a combined therapy.

        As used herein, the term "differentiation" refers
to a developmental process of the state of the complex parts
of organisms, such as cells, tissues, or organs and a process
25   in which a characteristic tissue or organ is formed. The
term "differentiation" is mainly used in embryology,
developmental biology, and the like. In organisms, various
tissues and organs are formed from divisions of a fertilized
ovum (a single cell) to an adult. At early developmental
30   stages (i.e., before cell division or after insufficient
cell division), each cell or cell group has no morphological
or functional feature and is not much distinguishable. Such
a   state   is   referred   to   as   "undifferentiated".

"Differentiation" may occur at the level of organs. A cell constituting an organ may develop into various cells or cell groups having different features. This phenomenon is also referred to as differentiation within an organ in the formation of the organ. Therefore, a synthetic tissue or three-dimensional structure of the present invention may comprise a tissue including differentiated cells.

When differentiation is required to produce a synthetic tissue of the present invention, the differentiation may be performed either before or after the organization of the cells.

As used herein, the terms "differentiation agent" and "differentiation promoting agent" are used interchangeably and refer to any agent which is known to promote differentiation of cells (e.g., chemical substances, temperature, etc.). Examples of such an agent include, but are not limited to, various environmental factors, such as temperature, humidity, pH, salt concentration, nutrients, metals, gas, organic solvent, pressure, chemical substances (e.g., steroids, antibiotics, etc.), and the like, or arbitrary combinations thereof. Representative examples of differentiation agents include, but are not limited to, cellular physiologically active substances. Representative examples of cellular physiologically active substances include, but are not limited to, DNA demethylating agents (e.g., 5-azacytidine, etc.), histone deacetylating agents (e.g., trichosanthin, etc.), intranuclear receptor ligands (e.g., retinoic acid (ATRA), vitamin D_3, T3, etc.), cell growth factors (e.g., activin, IGF-1, FGF, PDGF, TGF-β, BMP2/4, etc.), cytokines (e.g., LIF, IL-2, IL-6, etc.), hexamethylenebisacetoamides, dimethylacetoamides, dibutyl

- 76 -

cAMPs, dimethylsulfoxides, iododeoxyuridines, hydroxyl
ureas, cytosine arabinosides, mitomycin C, sodium lactate,
aphydicolin, fluorodeoxyuridine, polybren hexadimetrine
bromide, selenium, and the like.

Specific examples of differentiation agents are
described below. These differentiation agents may be used
singly or in combination.

A) Cornea: epidermal growth factor (EGF);

B) Skin (keratinocyte): TGF-β, FGF-7 (KGF: keratinocyte
growth factor), EGF;

C) Vascular endothelium: VEGF, FGF, angiopoietin;

D) Kidney: LIF, BMP, FGF, GDNF;

E) Heart: HGF, LIF, VEGF;

F) Liver: HGF, TGF-β, IL-6, EGF, VEGF;

G) Umbilical endothelium: VEGF;

H) Intestinal epithelium: EGF, IGF-1, HGF, KGF, TGF-β, IL-11;

I) Nerve: nerve growth factor (NGF), BDNF (brain-derived
neurotrophic factor), GDNF (glial-derived neurotrophic
factor), neurotrophin, IL-6, TGF-β, TNF;

J) Glia cell: TGF-β, TNF-α, EGF, LIF, IL-6;

K) Peripheral nerve cell: bFGF, LIF, TGF-β, IL-6, VEGF;

L) Lung (alveolar epithelium): TGF-β, IL-13, IL-1β, KGF, HGF;

M) Placenta: growth hormone (GH), IGF, prolactin, LIF, IL-1,
activin A, EGF;

N) Pancreatic epithelium: growth hormone, prolactin;

O) Pancreatic Langerhans' cells: TGF-β, IGF, PDGF, EGF,
TGF-β, TRH (thyrotropin);

P) Synovial cell: FGF, TGF-β (particularly, TGF-β1, TGF-β3);

Q) Osteoblast: BMP (particularly, BMP-2, BMP-4, BMP-7), FGF;

R) Chondroblast: FGF, TGF-β (particularly, TGF-β1, TGF-β3),
BMP (particularly, BMP-2, BMP-4, BMP-7), TNF-α, IGF;

S) Retinal cell: FGF, CNTF (cilliary neurotrophic factor);

T) Fat cell: insulin, IGF, LIF; and

U) Muscle cell: LIF, TNF-α, FGF.

5   As used herein, the term "osteogenesis" indicates
that any cell is caused to differentiate into a osteocyte.
It is known that osteogenesis is promoted in the presence
of dexamethasone, β-glycerophosphate, and ascorbic acid
2-phosphate. An osteogenic agent (BMP, (particularly,
10   BMP-2, BMP-4, BMP-7)) may be added to promote osteogenesis.
           As used herein, the term "chondrogenesis" refers to
differentiation of any cell into a chondrocyte. It is known
that chondrogenesis is promoted in the presence of pyrubic
acid, dexamethasone, ascorbic acid 2-phosphate, insulin,
15   transferrine, and selenious acid. An bone morphogenetic
protein (BMP, (particularly, BMP-2, BMP-4, BMP-7)), TGF-β
(particularly, TGF-β1 and TGF-β3), FGF, TNF-α and the like
.   may be added to promote chondrogenesis.

20   As used herein, the term "adipogenesis" refers to
differentiation of any cell into an adipocyte. It is known
that adipogenesis is promoted in the presence of insulin,
IGF, LIF, and ascorbic acid 2-phosphate.

25   As used herein, the terms "implant", "graft", and
"tissue graft" are used interchangeably, referring to
homologous or heterologous tissue or a cell group, or an
artificial material, which is inserted into a particular
site of a body and thereafter forms a part of the body.
30   Therefore, a synthetic tissue or three-dimensional structure
of the present invention can be used as an implant. Examples
of conventional grafts include, but are not limited to, organs
or portions of organs, blood vessels, blood vessel-like

tissue, heart, cardiac valves, pericardia, dura matter, joint
capsule, bone, cartilage, cornea, tooth, and the like.
Therefore, grafts encompass any one of these which is inserted
into an injured part so as to compensate for the lost portion.
5   Grafts include, but are not limited to, autografts,
allografts, and xenografts, which depend on the type of their
donor.

         As used herein, the term "autograft" (a tissue, a
10 cell, an organ, etc.) refers to a graft (a tissue, a cell,
an organ, etc.) which is implanted into the same individual
from which the graft is derived. As used herein, the term
"autograft" (a tissue, a cell, an organ, etc.) may encompass
a graft from a genetically identical individual (e.g. an
15 identical twin) in a broad sense. As used herein, the terms
"autologous" and "derived from a subject" are used
interchangeably. Therefore, the term "not derived from a
subject" in relation to a graft indicates that the graft
is not autologous (i.e., heterologous).
20

         As used herein, the term "allograft (a tissue, a cell,
an organ, etc.)" refers to a graft (a tissue, a cell, an
organ, etc.) which is transplanted from a donor genetically
different from, though of the same species, as the recipient.
25 Since an allograft is genetically different from the
recipient, the allograft (a tissue, a cell, an organ, etc.)
may elicit an immune reaction in the recipient. Examples
of such grafts (a tissue, a cell, an organ, etc.) include,
but are not limited to, grafts derived from parents (a tissue,
30 a cell, an organ, etc.). The synthetic tissue of the present
invention can be an allograft, which has been demonstrated
to have satisfactory therapeutic results. Attention should
be paid to the synthetic tissue of the present invention.

As used herein, the term "xenograft" (a tissue, a
cell, an organ, etc.) refers to a graft (a tissue, a cell,
an organ, etc.) which is implanted from a different species.
5   Therefore, for example, when a human is a recipient, a
porcine-derived graft (a tissue, a cell, an organ, etc.)
is called a xenograft (a tissue, a cell, an organ, etc.).

As used herein, "recipient" (acceptor) refers to an
10   individual which receives a graft (a tissue, a cell, an organ,
etc.) or implanted matter (a tissue, a cell, an organ, etc.)
and is also called "host". In contrast, an individual
providing a graft (a tissue, a cell, an organ, etc.) or
implanted matter (a tissue, a cell, an organ, etc.) is called
15   "donor" (provider).

With a synthetic tissue forming technique of the
present invention, a synthetic tissue derived from any cell
can be used. This is because a synthetic tissue (e.g.,
20   membranous tissues, organs, etc.) formed by the method of
the present invention can exhibit a desired function while
the tissue injury rate is maintained at a level which does
not interfere with the therapy (i.e., a low level).
Conventionally, tissues or organs are used as grafts without
25   modification. In contrast to this, the present invention
provides a tissue comprising three-dimensionally connected
cells. Such a synthetic three-dimensional tissue cannot be
achieved by conventional techniques, and therefore,
constitutes one significant effect of the present invention.
30

As used herein, the term "subject" refers to an
organism to which treatment of the present invention is
applied and is also referred to as "patient". A patient or

subject may be preferably a human.

Cells optionally used in a synthetic tissue, three-dimensional structure, or tissue graft of the present invention may be derived from a syngeneic origin (self origin), an allogenic origin (non-self origin), or a heterologous origin. In view of rejection reactions, syngeneic cells are preferable. If rejection reactions do not raise problems, allogenic cells may be employed. Cells which elicit rejection reactions can be employed by optionally treating the cells in a manner that overcomes rejection reactions. Procedures for avoiding rejection reactions are known in the art (see, for example, "Shin Gekagaku Taikei, Dai 12 Kan, Zoki Ishoku (Shinzo Ishoku · Hai Ishoku Gijutsuteki, Rinriteki Seibi kara Jisshi ni Mukete [New Whole Surgery, Vol. 12, Organ Transplantation (Heart Transplantation · Lung Transplantation From Technical and Ethical Improvements to Practice)" (Revised 3rd ed.), Nakayama Shoten]. Examples of such methods include, but are not limited to, a method using immunosuppressants or steroidal drugs, and the like. For example, there are currently the following immunosuppressants for preventing rejection reactions: "cyclosporine" (SANDIMMUNE/NEORAL); "tacrolimus" (PROGRAF); "azathioprine" (IMURAN); "steroid hormone" (prednine, methylprednine); and "T-cell antibodies" (OKT3, ATG, etc.). A method which is used worldwide as a preventive immunosuppression therapy in many facilities, is the concurrent use of three drugs: cyclosporine, azathioprine, and steroid hormone. An immunosuppressant is desirably administered concurrently with a pharmaceutical agent of the present invention. The present invention is not limited to this. An immunosuppressant may be administered before or after a regeneration/therapeutic method of the present

invention as long as an immunosuppression effect can be
achieved.

Cells used in the present invention may be derived
from any organism (e.g., vertebrates and invertebrates).
Preferably, cells derived from vertebrates are used. More
preferably, cells derived from mammals (e.g., primates,
rodents, etc.) are used. Even more preferably, cells derived
from primates are used. Most preferably, cells derived from
a human are used. Typically, cells from the same species
as the host are preferably used.

Examples of an affected portion of a subject treated
by a synthetic tissue of the present invention include, but
are not limited to, the heart suffering from a heart disease
(e.g., heart failure, ischemic heart diseases, myocardial
infarct,   cardiomyopathy,   myocarditis,   hypertrophic
cardiomyopathy, dilated hypertrophic cardiomyopathy, and
dilated cardiomyopathy); blood vessels in a pericardium
patch, infarcted myocardium lower and upper limbs; a joint
injury or denaturation; a cartilage injury or denaturation;
osteonecrosis;   meniscus   injury   or   denaturation;
intervertebral disk denaturation; ligament injury or
denaturation; a fracture; implantation to a patient having
a joint, cartilage, or bone having bone loss; an injured
cornea; and the like.

Tissues targeted by the present invention may be any
organ of an organism and may be derived from any organism.
Examples of organisms targeted by the present invention
include vertebrates and invertebrates.   Preferably,
organisms targeted by the present invention are mammals (e.g.,
primates, rodents, etc.).   More preferably, organisms

targeted by the present invention are primates.   Most
preferably, organisms targeted by the present invention are
humans.

5   As used herein, the term "flexibility" in relation
to a synthetic tissue refers to an ability to resist physical
stimuli from external environments (e.g., pressure).   A
synthetic tissue having flexibility is preferable when the
implantation site moves or deforms autonomously or by
10   external effects.

           As used herein, the term "extendibility and
contractibility" in relation to a synthetic tissue refers
to an ability to resist extending or contracting stimuli
15   from external environments (e.g., pulsation).   A synthetic
tissue having extendibility and contractibility is
preferable when the implantation site is subjected to
extending or contracting stimuli.   Examples of implantation
sites, which are subjected to extending or contracting
20   stimuli, include, but are not limited to, heart, muscle,
joint, cartilage, tendon, and the like.   In one embodiment,
extendibility and contractibility capable of withstanding
the pulsation motion of the heart may be required.

25   As used herein, the term "part" or "portion" refers
to any part or portion, tissue, cell, or organ in the body.
Examples of such parts, tissues, cells, and organs include,
but are not limited to, a portion which can be treated with
skeletal myoblasts, fibroblasts, synovial cells, stem cells,
30   and the like.   A marker specific to a portion may be any
parameter, such as a nucleic acid molecule (expression of
mRNA), a protein, an extracellular matrix, a specific
phenotype, a specific shape of a cell, or the like.   Therefore,

markers which are not specified herein may be used to identify
a synthetic tissue of the present invention as long as these
markers can indicate cells derived from a portion.
Representative examples of portions, but are not limited
5   to, portions of the heart other than the adult myocardium,
portions containing mesenchymal stem cells or cells derived
therefrom, other tissues, other organs, myoblasts (e.g.,
skeletal myoblasts), fibroblasts, synovial cells, and the
like.
10

For observing a cartilage tissue, following markers
can be used as index.

Sox9 (human: Accession No. NM_000346) is a marker
15   specific to a chondrocyte. The marker can be confirmed mainly
by observing the presence of mRNA (Kulyk WM, Franklin JL,
Hoffman LM. Sox9 expression during chondrogenesis in
micromass cultures of embryonic limb mesenchyme. Exp Cell
Res. 2000 Mar 15, 255(2):327-32.).
20

Col 2A1 (human: Accession No. NM_001844) is a marker
specific to a chondrocyte. The marker can be confirmed mainly
by observing the presence of mRNA (Kulyk WM, Franklin JL,
Hoffman LM. Sox9 expression during chondrogenesis in
25   micromass cultures of embryonic limb mesenchyme. Exp Cell
Res. 2000 Mar 15;255(2):327-32.).

Aggrecan (human: Accession No. NM_001135) is a
marker specific to a chondrocyte. The marker can be confirmed
30   mainly by observing the presence of mRNA (Kulyk WM, Franklin
JL, Hoffman LM. Sox9 expression during chondrogenesis in
micromass cultures of embryonic limb mesenchyme. Exp Cell
Res. 2000 Mar 15;255(2):327-32.).

Bone sialoprotein (human: Accession No. NM_004967)
is a marker specific to an osteoblast. The marker can be
confirmed mainly by observing the presence of mRNA (Haase
HR, Ivanovski S, Waters MJ, Bartold PM. Growth hormone
regulates osteogenic marker mRNA expression in human
periodontal fibroblasts and alveolar bone-derived cells.
J Periodontal Res. 2003 Aug;38(4):366-74.).

Osteocalcin (human: Accession No. NM_199173) is a
marker specific to an osteoblast. The marker can be confirmed
mainly by observing the presence of mRNA (Haase HR, Ivanovski
S, Waters MJ, Bartold PM. Growth hormone regulates
osteogenic marker mRNA expression in human periodontal
fibroblasts and alveolar bone-derived cells. J Periodontal
Res. 2003 Aug;38(4):366-74.).

GDF5 (human :Accession No. NM_000557) is a marker
specific to a ligament cell. The marker can be confirmed
mainly by observing the presence of mRNA (Woltman NM,
Hattersley G, Cox K, Celeste AJ, Nelson R, Yamaji N, Dube
JL, DiBlasio-Smith E, Nove J, Song JJ, Wozney JM, Rosen V.
Ectopic induction of tendon and ligament in rats by growth
and differentiation factors 5, 6, and 7, members of the
TGF-beta gene family. J Clin Invest. 1997 Jul
15;100(2):321-30.).

Six1 (human: Accession No. NM_005982) is a marker
specific to a ligament cell (Dreyer SD, Naruse T, Morello
R, Zabel B, Winterpacht A, Johnson RL, Lee B, Oberg KC. Lmx1b
expression during joint and tendon formation: localization
and evaluation of potential downstream targets. Gene Expr
Patterns. 2004 Jul;4(4):397-405.). The marker can be

confirmed mainly by observing the presence of mRNA.

Scleraxis (human :Accession No. BK000280) is a
marker specific to a ligament cell (Brent AE, Schweitzer
R, Tabin CJ. A somitic compartment of tendon progenitors.
Cell. 2003 Apr 18;113(2):235-48.). The marker can be
confirmed mainly by observing the presence of mRNA.

A "part other than the myocardium of an adult" and
a "part other than the heart of an adult" can be identified
using markers characteristic to cells derived from the
myocardium of an adult or the heart of an adult including
skeletal myoblasts, fibroblasts, synovial cells, stem cells,
or the like (hereinafter referred to as a "non-adult
myocardial marker" or a "non-adult heart ' marker",
respectively). If the marker is expressed by less than about
100%, preferably less than about 80%, more preferably less
than about 50%, even more preferably less than about 25%,
in some cases less than about 1%, the above-described parts
can be identified. Examples of such markers include, but
are not limited to, myosin heavy chain IIa, myosin heavy
chain IIb, myosin heavy chain IId (IIx), CD56, MyoD, Myf5,
myogenin, and the like. Therefore, non-adult myocardial
markers which are not specified herein may be used to identify
a synthetic tissue of the present invention as long as these
markers can indicate cells derived from parts other than
the myocardium of an adult. Also, non-adult heart markers
which are not specified herein may be used to identify a
synthetic tissue of the present invention as long as these
markers can indicate cells derived from parts other than
the heart of an adult.

Myosin   heavy   chain   IIa   (human: Accession

No. NM_017534; SEQ ID NOs. 1 and 2), myosin heavy chain IIb
(human: Accession No. NM_017533; SEQ ID NOs. 3 and 4), and
myosin heavy chain IId (IIx) (human: Accession No. NM_005963;
SEQ ID NOs. 5 and 6) are markers specific to myoblasts
(Havenith M.G., Visser R., Schrijvers-van Schendel J.M.,
Bosman F.T., "Muscle Fiber Typing in Routinely Processed
Skeletal Muscle With Monoclonal Antibodies", Histochemistry,
1990; 93(5):497-499). These markers can be confirmed mainly
by observing the presence of proteins. An antibody against
myosin heavy chain IIa, myosin heavy chain IIb, and myosin
heavy chain IId (IIx) is, for example, MY-32 available from
Sigma. This antibody is specific to skeletal muscles and
does not bind to myocardium (Webster C., Pavlath G.K., Parks
D.R., Walsh F.S., Blau H.M., Exp. Cell. Res., 1988 Jan;
174(1):252-65; and Havenith M.G., Visser R., Schrijvers-van
Schendel J.M., Bosman F.T., Muscle Fiber Typing in Routinely
Processed Skeletal Muscle with Monoclonal Antibodies,
Histochemistry, 1990, 93(5):497-499).

        CD56 (human: Accession No. U63041; SEQ ID NOs. 7 and
8) is a marker specific to myoblasts. This marker can be
confirmed mainly by observing the presence of mRNA.

        MyoD (human: Accession No. X56677; SEQ ID NOs. 9 and
10) is a marker specific to myoblasts. This marker can be
confirmed mainly by observing the presence of mRNA.

        Myf5 (human: Accession No. NM_005593; SEQ ID NOs. 11
and 12) is a marker specific to myoblasts. This marker can
be confirmed mainly by observing the presence of mRNA.

        Myogenin (human: Accession No. BT007233; SEQ ID
NOs. 13 and 14) is a marker specific to myoblasts. This

marker can be confirmed mainly by observing the presence
of mRNA.

In other embodiments, other markers specific to other
tissues can be utilized. Examples of such markers include,
but are not limited to, Oct-3/4, SSEA-1, Rex-1, Otx2, and
the like for embryonic stem cells; VE-cadherin, Flk-1, Tie-1,
PECAM1, vWF, c-kit, CD34, Thy1, Sca-1, and the like for
endothelial cells; skeletal muscle α actin in addition to
the above-described markers for skeletal muscles; Nestin,
Glu receptor, NMDA receptor, GFAP, neuregulin-1, and the
like for nerve cells; c-kit, CD34, Thy1, Sca-1, GATA-1, GATA-2,
FOG, and the like for hematopoietic cells.

As used herein, the term "derived" in relation to
cells means that the cells are separated, isolated, or
extracted from a cell mass, tissue, or organ in which the
cells have been originally present, or that the cells are
induced from stem cells.

As used herein, the term "applicable to heart" means
that the heart applied has an ability to pulsate. A tissue
applicable to heart has strength such that the tissue can
withstand dilation and contraction of the pulsating heart.
Here, applicability to the heart includes applicability to
the myocardium. Applicability to heart may be determined
by confirming that a recipient having an implanted graft
survives.

As used herein, the term "three-dimensional
structure" refers to an object which comprises cells having
intracellular intergration or alignment and extends
three-dimensionally, particularly matrices are oriented

- 88 -

three-dimensionally   and   cells   are   arranged
three-dimensionally.

As used herein, the term "biological integration"
in relation to the relationship between biological entities
such as cells means that there is certain interaction between
the biological entities.   Examples of such interaction
includes, but are not limited to, interaction via biological
molecules (e.g., extracellular matrix), interaction via
signal transduction, electrical interaction (electrical
integration, such as synchronization of electrical signals
or the like), and the like. Biological integration includes
biological integration in a synthetic tissue and biological
integration of a synthetic tissue with its surroundings (e.g.,
surrounding tissues and cells after implantation, etc.).
In order to confirm interactions, an assay appropriate to
a characteristic of the interaction is employed. In order
to confirm physical interactions via biological molecules,
the strength of a synthetic tissue, a three-dimensional
structure, or the like is measured (e.g., a tensile test).
In order to confirm interaction via signal transduction,
gene expression or the like is investigated. In order to
confirm electrical interactions, the electric potential of
a synthetic tissue, a three-dimensional structure, or the
like is measured to determine whether or not the electric
potential is propagated with constant waves. In the present
invention, biological integration is provided in all three
dimensions. Preferably, there is biological integration
substantially   uniformly   in   all   directions   in   a
three-dimensional space.   In another embodiment, the
synthetic tissue, a three-dimensional structure, and the
like, which has substantially uniform two-dimensional
biological integration and slightly weaker biological

- 89 -

integration in the third dimension, may be employed.
Biological integration via an extracellular matrix can be
confirmed based on the degree of staining by staining the
extracellular matrix. As a method for observing biological
integration in vivo, there is an integration experiment using
cartilage. In this experiment, a surface of the cartilage
is removed and digested with chondroitinase ABC (Hunziker
E.B. et al., J. Bone Joint Surg. Am., 1996 May; 78(5): 721-33).
Thereafter, a tissue of interest is implanted onto a cut
surface, followed by culturing for about 7 days. The
subsequent integration is observed (Figure 23). It will be
understood that a capability to adhere to surrounding cells
can be determined with the above-described cartilage
experiment.

A synthetic tissue, three-dimensional structure, or
the like of the present invention may be provided using known
preparation methods, as a pharmaceutical product, or
alternatively, as an animal drug, a quasi-drug, a marine
drug, a cosmetic product, and the like.

Animals targeted by the present invention include
any organism as long as it has organs (e.g., animals (e.g.,
vertebrates, invertebrate)). Preferably, the animal is a
vertebrate (e.g., Myxiniformes, Petronyzoniformes,
Chondrichthyes, Osteichthyes, amphibian, reptilian, avian,
mammalian, etc.), more preferably mammalian (e.g.,
monotremata, marsupialia, edentate, dermoptera, chiroptera,
carnivore, insectivore, proboscidea, perissodactyla,
artiodactyla, tubulidentata, pholidota, sirenia, cetacean,
primates, rodentia, lagomorpha, etc.). Illustrative
examples of a subject include, but are not limited to, animals,
such as cattle, pigs, horses, chickens, cats, dogs, and the

like. More preferably, primates (e.g., chimpanzee, Japanese monkey, human, etc.) are used. Most preferably, a human is used. This is because there is limitation to implantation therapies.

When the present invention is used as a pharmaceutical agent, it may further comprise a pharmaceutically acceptable carrier or the like. A pharmaceutically acceptable carrier contained in a medicament of the present invention includes any material known in the art.

Examples of such a pharmaceutically acceptable carrier include, but are not limited to, antioxidants, preservatives, colorants, flavoring agents, diluents, emulsifiers, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, agricultural or pharmaceutical adjuvants, and the like.

The amount of a pharmaceutical agent (e.g., a synthetic tissue, a pharmaceutical compound used in conjunction therewith, etc.) used in the treatment method of the present invention can be easily determined by those skilled in the art with reference to the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex, and case history, the form or type of the cell, and the like. The frequency of the treatment method of the present invention applied to a subject (or patient) is also determined by those skilled in the art with respect to the purpose of use, target disease (type, severity, and the like), the patient's age, weight, sex, and case history, the progression of the therapy, and the like. Examples of the frequency include once per day to several months (e.g., once per week to once per month). Preferably, administration

is performed once per week to month with reference to the progression.

As used herein, the term "administer" in relation
to a synthetic tissue, three-dimensional structure, or the
like of the present invention or a pharmaceutical agent
comprising it, means that they are administered singly or
in combination with other therapeutic agents. A synthetic
tissue of the present invention may be introduced into therapy
sites (e.g., impaired heart, etc.) by the following methods,
in the following forms, and in the following amounts.
Examples of the introduction methods include, but are not
limited to, direct attachment, suture after attachment,
insertion, and the like. For example, a synthetic tissue
and a three-dimensional structure of the present invention
may be applied by the above-described methods to an impaired
site of ischemic myocardial tissue caused by myocardial
infarct, angina pectoris, or the like. Combinations may be
administered either concomitantly (e.g., as an admixture),
separately but simultaneously or concurrently; or
sequentially. This includes presentations in which the
combined agents are administered together as a therapeutic
mixture, and also procedures in which the combined agents
are administered separately but simultaneously (e.g., a
synthetic tissue or the like is directly provided by operation,
while other pharmaceutical agents are provided by intravenous
injection). "Combination" administration further includes
the separate administration of one of the compounds or agents
given first, followed by the second.

As used herein, the term "reinforcement" means that
the function of a targeted part of an organism is improved.

          As used herein, the term "instructions" describe how
to handle reagents, usage, a preparation method, a method
of producing a synthetic tissue, a method of administering
a medicament of the present invention, a method for diagnosis,
5   or the like for persons who administer, or are administered,
the medicament or the like or persons who diagnose or are
diagnosed (e.g, physicians, patients, and the like). The
instructions describe a statement indicating an appropriate
method for administering a diagnostic, a medicament, or the
10   like of the present invention. The instructions are prepared
in accordance with a format defined by an authority of a
country in which the present invention is practiced (e.g.,
Health, Labor and Welfare Ministry in Japan, Food and Drug
Administration (FDA) in the U.S., and the like), explicitly
15   describing that the instructions are approved by the
authority. The instructions are so-called package insert
and are typically provided in paper media. The instructions
are not so limited and may be provided in the form of electronic
media (e.g., web sites, electronic mails, and the like
20   provided on the Internet).

          As used herein, the term "extracellular matrix
synthesis promoting agent" or "ECM synthesis promoting agent"
refers to an agent which promotes the production of an
25   extracellular matrix of a cell. In the present invention,
when an ECM synthesis promoting agent is added to a cell
sheet, an environment which promotes self-contraction of
cells after a cell sheet is detached from a culture container.
The sheet is biologically organized in three-dimensional
30   directions. Examples of such an agent representatively
include agents capable of promoting the secretion of an
extracellular matrix (e.g., TGF-β1, TGF-β3, etc.). Examples
of an ECM synthesis promoting agent representatively include,

but are not limited to, TGF-β1, TGF-β3, ascorbic acid,
ascorbic acid 2-phosphate, or a derivative or salt thereof.
Preferably, an ECM synthesis promoting agent may be
preferably a component of an extracellular matrix of a part
targeted by application and/or a component(s) capable of
promoting the secretion of an extracellular matrix in an
amount similar thereto. When an ECM synthesis promoting
agent comprises a plurality of components, the components
may be components of an extracellular matrix of a part targeted
by application and/or components capable of promoting the
secretion of an extracellular matrix in an amount similar
thereto.

As used herein, the term "ascorbic acid or a
derivative thereof" includes ascorbic acid and an analog
thereto (e.g., ascorbic acid 2-phosphate, ascorbic acid
1-phosphate, etc.), and a salt thereof (e.g., sodium salt,
magnesium salt, etc.). Ascorbic acid is preferably, but is
not limited to, an L-isomer.

(Description of the Preferred Embodiments)
Hereinafter, preferable embodiments of the present
invention will be described. The following embodiments are
provided for a better understanding of the present invention
and the scope of the present invention should not be limited
to the following description. It will be clearly appreciated
by those skilled in the art that variations and modifications
can be made without departing from the scope of the present
invention with reference to the specification.

In an aspect of the present invention, the synthetic
tissue and complex of the present invention is free of injury
caused by a protein degrading enzyme, such as,

representatively, dispase, trypsin, or the like, during
culture. Therefore, the synthetic tissue and complex, which
is detached from the base material, can be recovered as a
cell mass holding proteins between cells (e.g., an
5   extracellular matrix) and having a certain level of strength.
The synthetic tissue and complex also retain intact functions,
such as an intracellular linking manner, alignment, and the
like. When typical protein degrading enzymes (e.g., trypsin,
etc.) are used to detach the three-dimensional structure
10   or synthetic tissue, substantially no cell-to-cell link or
cell-to-extracellular matrix link are retained, so that cells
are individually separated. Among these protein degrading
enzymes, dispase destroys basement membrane-like proteins
between cells and base materials substantially completely.
15   In this case, however, the resultant three-dimensional
structure or synthetic tissue has weak strength. In contrast,
the three-dimensional structure or synthetic tissue of the
present invention can both substantially completely retain
each of the desmosome structure and the basement
20   membrane-like protein, resulting in the above-described
various effects.

In the method of the present invention, the period
of time required for culture may be determined depending
25   on the application of the synthetic tissue or
three-dimensional structure. In order to detach and recover
the cultured synthetic tissue or three-dimensional structure
from the support material, the cultured synthetic tissue
or three-dimensional structure is detached directly, or with
30   macromolecular membrane being attached thereto. Note that
the synthetic tissue or three-dimensional structure may be
detached in culture medium in which cells have been cultured,
or alternatively, in other isotonic solutions. Such

- 95 -

solutions may be selected depending on the purpose. When
a monolayer cell sheet is prepared, examples of the
macromolecular membrane, which is optionally attached to
the cell sheet or three-dimensional structure, include, but
5   are not limited to, hydrophilized polyvinylidene difluoride
(PVDF), polypropylene, polyethylene, cellulose and
derivatives thereof, chitin, chitosan, collagen, paper (e.g.,
Japan paper, etc.), urethane, net-like or stockinette-like
macromolecular materials (e.g., spandex, etc.), and the like.
10 When a net-like or stockinette-like macromolecular material
is employed, the synthetic tissue or complex has a higher
degree of freedom, so that the contraction/relaxation
function thereof can be increased. A method for producing
the synthetic tissue or three-dimensional structure
15 comprising cells of the present invention is not particularly
limited. For example, the synthetic tissue or
three-dimensional structure of the present invention can
be produced by utilizing the above-described cultured cell
sheet attached to a macromolecular membrane.
20

In order to detach and recover the synthetic tissue
or complex with a high yield from the cell culture support,
the cell culture support is tapped or shaken, or the medium
is stirred with a pipette. These procedures may be performed
25 singly or in combination. In addition, the synthetic tissue
or complex may be detached and recovered by deforming the
base of the culture container or rinsing the container with
isotonic solution or the like. By stretching the synthetic
tissue or complex in a specific direction after being detached
30 from the base material, the complex is provided with alignment.
Stretching may be performed by using a tensile device (e.g.,
Tensilon, etc.), or simply forceps, or the like. A stretching
method is not particularly limited. By providing alignment,

it is possible to confer directionality to the motion of
the cell sheet or complex itself. Therefore, for example,
it is possible to allow the synthetic tissue or complex to
move in accordance with the motion of a specific organ. The
synthetic tissue or complex can be efficiently applied to
organs.

The thus-obtained synthetic tissue or complex cannot
be obtained by conventional techniques.

The synthetic tissue and the complex according to
the present invention includes an abundance of adhesion
molecules such as extracellular matrix which may include
collagen (types I, III, etc.), vironectin, and fibronectin,
and can be accepted by the surrounding tissue. Thus,
implanted cells can be stably accepted by the implantation
site. In conventional cell implantation, it was difficult
for cells to be stably accepted by the implantation site
not only in cells implantation without a scaffold, but also
in cell implantation using an additional stabilizing
treatment (e.g., sewing of a patch, scaffold, etc.). However,
use of the present invention facilitates stabilization.
When only cells are used, reinforcement by another tissue,
fixing scaffold, or the like is necessary. According to the
present invention, without requiring such means, cells which
may have pluripotency included in the synthetic tissue or
complex can be stably accepted by the implantation portion
without an additional fixing means.

(Preparation of synthetic tissue using an ECM
synthesis promoting agent)

In another aspect, the present invention provides
a method for producing a synthetic tissue. The method for

producing a synthetic tissue comprises the steps of:
A) providing a cell; B) placing the cell in a container
containing a cell culture medium including an ECM synthesis
promoting agent, wherein the container has a base with an
area sufficient to accommodate a desired size of the synthetic
tissue; and C) culturing the cell in the container for a
period of time sufficient to form the synthetic tissue having
the desired size.

The above-described cell may be any cell. A method
for providing a cell is well known in the art. For example,
a tissue is extracted and cells are isolated from the tissue.
Alternatively, cells are isolated from body fluid containing
blood cells or the like. Alternatively, a cell line is
prepared in an artificial culture. The present invention
is not limited to this. Cells used herein may be any stem
cells or differentiated cells, particularly including
myoblasts, mesenchymal stem cells, adipocytes, synovial
cells, bone marrow cells, and the like. Examples of
mesenchymal stem cells used herein include adipose
tissue-derived stem cells, bone marrow-derived stem cells,
and the like.

The method for producing a synthetic tissue of the
present invention employs a cell culture medium containing
an ECM synthesis promoting agent. Examples of such an ECM
synthesis promoting agent include, but are not limited to,
ascorbic acid or a derivative thereof, ascorbic acid
1-phosphate, ascorbic acid 2-phosphate, L-ascorbic acid,
and the like.

The cell culture medium used in the present invention
may be any medium which allows a cell of interest to grow.

- 98 -

Examples of such a medium include, but are not limited to, DMEM, MEM, F12, DME, RPMI1640, MCDB104, 199, MCDB153, L15, SkBM, Basal medium, and the like which are supplemented with glucose, FCS (fetal calf serum), antibiotics (penicillin,
5   streptomycin, etc.) as appropriate.

The container used in the present invention may be any container typically used in the art which has a base with an area sufficient to accommodate a desired size of
10 the synthetic tissue. Examples of such a container include, but are not limited to, petri dishes, flasks, mold containers, and the like, and preferably containers having a large area of the base (e.g., at least 1 cm^2). The material of the container may be any material and include, but are not limited
15 to, glass, plastic (e.g., polystyrene, polycarbonate, etc.), silicone, and the like.

In a preferable embodiment, the method for producing a synthetic tissue according to the present invention further
20 comprises detaching a produced synthetic tissue. As used herein, the term "detach" indicates that after a synthetic tissue of the present invention is formed in a container, the synthetic tissue is removed from the container. The detachment can be achieved by, for example, physical means
25 (e.g., pipetting of medium, etc.), chemical means (addition of a substance), or the like. In the present invention, a synthetic tissue can be detached by providing a stimulus around the synthetic tissue by physical means or chemical means, but not by aggressive means (e.g., treatment with
30 a protein degrading enzyme, etc.) to the synthetic tissue. Thus, the present invention provides ease of handling, which cannot be conventionally achieved, and the resulting synthetic tissue is substantially intact, resulting in a

- 99 -

high-performance implant.

In a preferable embodiment, the present invention further comprises detaching cells which construct a synthetic tissue. In a more preferable embodiment, the detaching step includes applying a stimulus for contracting a synthetic tissue, including a physical stimulus (e.g., pipetting, etc.). Such a physical stimulus is not directly applied to the produced synthetic tissue. This is a preferable feature of the present invention. Since a physical stimulus is not directly applied to a synthetic tissue, it is possible to suppress damage to the synthetic tissue. Alternatively, the detaching step includes chemical means, such as adding an actin regulatory agent. Such an actin regulatory agent includes a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents. Examples of actin depolymerizing agents include, but are not limited to, ADF (actin depolymerizing factor), destrin, depactin, actophorin, cytochalasin, NGF (nerve growth factor), and the like. Examples of actin polymerizing agents include, but are not limited to, LPA (lysophosphatidic acid), insulin, PDGFm, chemokine, TGF b, and the like.

Though not wishing to be bound by any theory, these actin regulatory agents may cause actomyocin-based cytoskeleton to contract or extend, thereby regulating contraction and extension of a cell itself. As a result, a synthetic tissue itself may be promoted to or inhibited from being detached from the base of a container.

In another embodiment, the synthetic tissue and complex of the present invention are characterized in that

they are produced from cells which are cultured in monolayer
culture. Despite monolayer culture, synthetic tissues
having various thicknesses can be constructed. This is an
unexpected effect. Conventionally, for example, a thick
5   tissue cannot be constructed without using a multilayer
structure when a temperature responsive sheet or the like
is used. The present invention is the first to achieve a
method for constructing a three-dimetional structure, which
does not require a scaffold and can construct the contractile
10   organization including ten or more layers. A typical cell
implantation method which does not employ a scaffold is a
cell sheet engineering technique utilizing a temperature
sensitive culture dish disclosed by Kushida A., Yamato M.,
Konno C., Kikuchi A., Sakurai Y., Okano T., J. Biomed. Mater.
15   Res., 45:355-362, 1999. The technique has won international
recognition as an original technique. However, this cell
sheet technique has a problem in that a single sheet is weak
in many cases, and requires modification such as layering
sheets for obtaining the strength resistant to an surgical
20   operation such as implantation.

A cell/matrix complex developed by the present
invention does not require a temperature sensitive culture
dish unlike the cell sheet technique. The cell/matrix
25   complex is easy to form into a contractile three-dimensional
tissue. There is no technique in the world other than the
present invention, which can produce a contractile
three-dimensional complex having 10 or more layers without
using so-called feeder cells, such as rodent stroma cells,
30   after approximately three weeks. By adjusting conditions
for matrix production of the synovial cell, it is possible
to produce a complex having a strength which allows surgical
manipulation, such as holding or transferring the complex,

without a special instrument. Therefore, the present invention is an original, epoch-making technique in the world for reliably and safely perform cell implantation.

5   In a preferable embodiment, the ECM synthesis promoting agent used in the method for producing a synthetic tissue of the present invention includes ascorbic acid 2-phosphate (Hata R., Senoo H., J. Cell Physiol., 1989, 138(1):8-16). In the present invention, by adding a certain
10   amount or more of ascorbic acid 2-phosphate, it is possible to promote production of an extracellular matrix, so that the resultant synthetic tissue or complex is made strong to become easy to be detached. Thereafter, self contraction is elicited by applying a stimulus for detachment. Hata et
15   al. do not report that, after adding such an ascorbic acid and culturing, a tissue becomes strong and obtains a property to be easy to be detached. Though not wishing to be bound by any theory, a significant difference is that Hata et al. used a significantly different cell density. Hata et al.
20   does not suggest an effect of making a tissue rigid. Such an effect that the tissue is made rigid, an effect of contraction, and an effect that the tissue becomes easy to be detached are first found in the present invention. The synthetic tissue according to the present invention is
25   recognized to be totally different from the synthetic tissue which has been fabricated conventionally at least on the point that it is produced through the process of making rigid, contraction, and detachment.

30   Conraction when the culture is detached and promotion in constructing a three-dimensional structure, a contractile three-dimensional tissue, or the like are suprising effects. Such effects have not been reported conventionally.

In a preferable embodiment, ascorbic acid 2-phosphate used in the present invention typically has a concentration of at least 0.01 mM, preferably at least 0.05 mM, more preferably at least 0.1 mM, even more preferably at least 0.2 mM, still more preferably at least 0.5 mM, and still even more preferably 1.0 mM. Herein, any concentration of 0.1 mM or higher may be employed. However, there may be an aspect in which a concentration of 10 mM or lower is desired.

In a certain preferable embodiment, the ECM synthesis promoting agent of the present invention includes ascorbic acid 2-phosphate or a salt thereof, and L-ascorbic acid or a salt thereof.

In a preferable embodiment, after the culturing step, the synthetic tissue production method of the present invention further comprises, detaching the synthetic tissue and allowing the synthetic tissue to perform self contraction. The detachment can be accelerated by applying a physical stimulus (e.g., application of shear stress, pipetting, deformation of the container, etc.). Self-contraction naturally takes place when a stimulus is applied after the detachment. When a chemical stimulus is applied, self-contraction and detachment occurs simultaneously. By self-contraction, biological integration is accelerated particularly in the third dimension (the direction perpendicular to the two-dimensional directions in the case of tissue on a sheet). Therefore, a synthetic tissue of the present invention may have a three-dimensional structure.

In a synthetic tissue production method of the present

invention, the sufficient time preferably means at least
3 days, though it varies depending on the application of
a synthetic tissue of interest. An exemplary period of time
is 3 to 7 days.

5

    In another embodiment, the synthetic tissue
production method of the present invention may further
comprise causing a synthetic tissue to differentitate. By
differentiation, the synthetic tissue can have a form closer

10 to that of a desired tissue. An example of such
differentiation is, but is not limited to, chondrogenesis
and osteogenesis. In a preferable embodiment, osteogenesis
may be performed in medium containing dexamethasone,
β-glycerophosphate, and ascorbic acid 2-phosphate. More

15 preferably, bone morphogenetic proteins (BMPs) are added.
This is because such BMP-2, BMP-4, and BMP-7 proteins promote
osteogenesis.

    In another embodiment, a method of producing the
20 synthetic tissue of the present invention is a process of
differentiating   a   synthetic   tissue.   A   form   of
differentiation includes performing a differentiation of
cartilage. In the preferable embodiment, chondrogenesis is
performed in a medium including pyruvic acid, dexamethasone,

25 ascorbic   acid   2-phosphate,   insulin,   transferrin,   and
selenious   acid.   More   preferably,   bone   morphogenetic
proteins (such as BMP-2, BMP-4, BMP-7), transforming growth
factors (such as TGF-β1, TGF-β3) are added. This is because
such BMPs promote chondrogenesis.

30

    An important point in the present invention is that
it is possible to fabricate a tissue having a pluripotency
into various differentiated cells such as bone, cartilage,

and the like. Conventionally, differentiation into a
cartilage tissue is difficult in other synthetic tissues
which are scaffold-free. If a certain size is required,
conventionally, it was necessary to coculture with a
5   scaffold, construct a three-dimensional structure, and add
a chondrogenesis medium. Conventionally, scaffold-free
differentiation into cartilage was difficult. The present
invention is the first to enable differentiation into
cartilage in a synthetic tissue. This is not an effect which
10   has not been obtained conventionally, and is a characteristic
effect of the present invention. In a treatment which aims
to regenerate a tissue, a method for performing a treatment
efficiently and safely by using a tissue of sufficient size
without a scaffold was difficult. The present invention
15   achieves a significant effect on this point. Particularly,
the present invention is significant on the point that it
becomes possible to easily manipulate differentiated cells
such as cartilage, which has been impossible conventionally.
Conventionally, for example, cells can be collected to a
20   pellete shape and the aggregation of cells can be
differentiated to obtain a tissue of about 2 mm^3. For
obtaining a tissue larger than this size, it was necessary
to use a scaffold.

25   The differentiation step in synthetic tissue
production of the present invention may be performed before
or after providing cells.

            In the present invention, primary culture cells can
30   be used. The present invention is not limited to this.
Subcultured cells (e.g., three or more passages) can also
be used. Preferably, when subculture cells are used, the
cells are preferably of four passages or more, more preferably

of 5 passages or more, and even more preferably of 6 passages
or more. The upper limit of cell density is increased with
an increase in the number of passages within a certain range.
This is because a denser synthetic tissue can be produced.
5   The present invention is not limited to this. It seems that
a certain range of passages (e.g., 3 to 8 passages) are
preferable.

     In the present invention, the cells are preferably
10   provided at a cell density of 5.0×10^4/cm^2 or more. The present
invention is not limited to this. This is because a higher
cell density can provide a synthetic tissue having a greater
strength. It will be understood that the lower limit of the
cell density may be lower than the above-described density.
15   It will also be understood that those skilled in the art
can define the lower limit based on the present specification.

     In one embodiment of the present invention, for
example, a myoblast, a synovial cell, an adipocyte, and a
20   mesenchymal stem sell (e.g., derived from adipose tissue
or bone marrow) can be used. The present invention is not
limited to this. These cells can be applied to, for example,
a heart, a bone, a cartilage, a tendon, a ligament, a joint,
a meniscus, and the like.

25

     (Synthetic tissue and complex)
     In another aspect, the present invention provides
a functional synthetic tissue or complex. The functional
synthetic tissue of the present invention is herein an
30   implantable synthetic tissue. Attempts have been
heretofore made to produce synthetic tissues by cell culture.
However, there were no synthetic tissues suitable for
implantation in terms of size, strength, physical injuries

when it is detached from a culture container, or the like.
The present invention provides a tissue culture method in
which cells are cultured in the presence of an ECM synthesis
promoting agent as described above, so that there is no problem
5   in terms of size, strength, and the like and there is no
difficulty in detaching tissues. An implantable synthetic
tissue is provided only after such a tissue culture method
is achieved.

10   Another aspect of the present invention provides
cells, and a complex including factors derived from the cells.
Herein, it is recognized that, preferably, the complex
substantially comprises cells, and the factors derived from
the cells. Herein, the complex of the present invention is
15   provided for reinforcing, repairing, or regenerating a part
of an organism.

          As used herein, the term "complex" means that cells
and other components are integrated into a complex by some
20   kind of interactivity. Therefore, the complex of the present
invention often has an appearance like a synthetic tissue,
and it is recognized that the meaning of the term "complex"
overlaps with what is referred to by a synthetic tissue.

25   The present invention provides a scaffold-free
synthetic tissue or complex. A therapeutic method and a
therapeutic agent for providing an excellent condition after
implantation can be obtained by providing such a
scaffold-free synthetic tissue.
30
          The scaffold-free synthetic tissue of the present
invention solves a long outstanding problem with biological
formulations, which is attributed to contamination of the

scaffold itself. Despite the absence of a scaffold, the therapeutic effect is comparable with, or more satisfactory than, conventional techniques.

5   In addition, when a scaffold is used, the alignment of implanted cells in the scaffold, the cell-to-cell adhesion, the in vivo alteration of the scaffold itself (eliciting inflammation), the acceptance of the scaffold by the recipient tissue, and the like become problematic.
10   These problems can be solved by the present invention.

          The synthetic tissue and the complex of the present invention are also self-organized, and have biological integration inside thereof. Also in this point, the present
15   invention is distinguished from conventional cell therapies.

          The synthetic tissue and the complex of the present invention are easily used to form a three-dimensional structure, and is thus easy to be designed into a desired
20   form. The versatility of the synthetic tissue and the complex of the present invention should be noted.

          The synthetic tissue and the complex of the present invention have biological integration with recipient
25   tissues, such as surrounding tissues, cells, and the like. Therefore, the post-operational acceptance is satisfactory, and cells are reliably supplied to a local site, for example. An effect of the present invention is that the satisfactory biological integration capability allows the formation of
30   a tissue complex with another synthetic tissue or the like, resulting in a complicated therapy.

          Another effect of the present invention is that

differentiation can be induced after the synthetic tissue
or the complex is provided. Alternatively, differentiation
is induced before providing a synthetic tissue and/or a
complex, and thereafter, the synthetic tissue and/or the
5   complex are formed.

Another effect of the present invention is that the
cell implantation of the present invention provides a
satisfactory replacement ability and a comprehensive supply
10   of cells for covering an implanted site, compared to
conventional cell-only implantation and sheet implantation.

The present invention provides an implantable
synthetic tissue. The above-described features and effects
15   of the present invention become it possible to treat a site
which cannot be considered as an implantation site for
conventional synthetic products. The present invention
makes it possible to provide a synthetic tissue or a
three-dimensional structure using not only a heart muscle
20   but also cells derived from other parts. The synthetic tissue
of the present invention has biological integration and
actually works in implantation therpies. The synthetic
tissue is first provided by the present invention, but is
not provided by conventional techniques.
25

In addition, the present invention provides medical
treatment which provides a therapeutic effect by filling,
replacing, and/or covering an affected portion.

30   In addition, when the synthetic tissue of the present
invention is used in combination with another synthetic
tissue (e.g., an artificial bone made of hydroxyapatite,
a microfibrous collagen medical device, etc.), the synthetic

tissue of the present invention is biologically integrated
with the other synthetic tissue, so that the acceptance of
the synthetic tissue can be improved to an extent which is
not conventionally expected.

An extracellular matrix or a cell adhesion molecule,
such as fibronectin, vitronectin, or the like, is distributed
throughout the synthetic tissue of the present invention.
In the cell sheet engineering, a cell adhesion molecule is
localized on a surface of culture cells which is attached
to a culture dish. In the sheet of the cell sheet engineering,
cells are major components of the sheet. The sheet is nearly
a mass of cells, on the bottom surface of which an adhesion
molecule (glue) is added. The synthetic tissue of the present
invention is a real "tissue" such that an extracellular matrix
wraps cells. Thus, the present invention is significantly
distinguished from conventional techniques.

A cell implanting method without a scaffold has been
reported by Kushida A., Yamato M., Konno C., Kikuchi A.,
Sakurai Y., Okano T., J. Biomed. Mater. Res., 45:355-362,
1999, in which a cell sheet is produced using a temperature
sensitive culture dish. Such a cell sheet engineering
technique is internationally appraised due to its originality.
However, a single sheet obtained by this technique is fragile.
In order to obtain the strength that can withstand surgical
manipulation, such as implantation, a plurality of sheets
need to be assembled, for example. Such a problem is solved
by the present invention.

A cell/matrix complex developed by the present
invention does not require a temperature sensitive culture
dish unlike the cell sheet technique. The cell/matrix

- 110 -

complex is easily formed into a contractile three-dimensional tissue. There is no technique in the world other than the present invention, which can produce a contractile three-dimensional complex having 10 or more layers without using so-called feeder cells, such as rodent stroma cells, after approximately three weeks. By adjusting conditions for matrix production of the synovial cell, it is possible to produce a complex having a strength which allows surgical manipulation, such as holding or transferring the complex, without a special instrument. Therefore, the present invention is an original, epoch-making technique in the world for reliably and safely performing cell implantation.

In a preferable embodiment, the synthetic tissue of the present invention has a biological integration capability to the surroundings. As used herein the term "surroundings" typically means surroundings to be implanted, and examples thereof include tissues, cells and the like. The biological integration capability with surrounding tissues, cells, and the like can be confirmed by, for example, photomicrograph, physical test, staining of a biological marker, or the like. Conventional synthetic tissues have a low affinity for adjacent tissues in which they are implanted. It was not even assumed that conventional synthetic tissues have the biological integration capability. Conventional synthetic tissues depend on a regeneration capability of an organism, and serves as a temporary solution until autologous cells gather and regenerate. These conventional synthetic tissues are not intended to for a permanent use. Therefore, the synthetic tissue of the present invention should be contemplated as an implantation treatment in the true sense. The biological integration capability referred to by in the present invention preferably includes an adhesion capability

to surrounding cells. Such an adhesion capability can be
measured by an in vitro culturing assay (see Figure 23) with
a tissue section (e.g., a cartilage section).

As used herein, the term "disease" to be treated by
5   the present invention refers to any disease accompanying
degeneration, necrosis, injury or the like, and examples
thereof including, osteoarthritis, osteochondral injury,
intractable fracture, osteonecrosis, cartilage injury,
meniscus injury, ligament injury, tendon injury, cartilage
10 degeneration, meniscus degeneration, intervertebral disk
denaturation, ligament degeneration, or tendon degeneration,
or any heart diseases having an injured tissue. Examples
of such heart diseases include heart failure, intractable
heart failure, myocardial infarct, cardiomyopathy, dilated
15 cardiomyopathy, hypertrophic cardiomyopathy, dilated phase
hypertrophic cardiomyopathy, and the like. The combined
therapy of the present invention may be applied to a
regeneration of an injury in an organ other than a heart,
as long as regeneration of a tissue injury is the goal. In
20 a specific embodiment, a disease to be treated by the method
of the present invention is intractable heart failure.

As used herein, the term "prophylaxis" or
"prevention" in relation to a certain disease or disorder
25 refers to a treatment which keeps such a condition from
happening before the condition is caused, or causes the
condition to occur at a reduced level or to be delayed.

As used herein, the term "therapy" in relation to
30 a certain disease or disorder means that when such a condition
occurs, such a disease or disorder is prevented from
deteriorating, preferably is retained as it is, more
preferably is diminished, and even more preferably

extinguished. As used herein, the term "radical therapy"
refers to a therapy which eradicates the root or cause of
a pathological process. Therefore, when a radical therapy
is made for a disease, there in principle is no recurrence
5 of the disease.

       As used herein, the term "prognosis" is also referred
to as "prognostic treatment". The term "prognosis" in
relation to a certain disease or disorder refers to a diagnosis
10 or treatment of such a condition after a therapy.

       In a preferable embodiment, the synthetic tissue or
complex of the present invention has a three-dimensional,
biological integration. As described in other portions of
15 the specification, examples of biological integration
include, but are not limited to, physical integration or
connection via extracellular matrices, electrical
integration, and the like. Particularly, in a preferable
embodiment including the cells, it is important that
20 extracellular matrix in a tissue is biologically organized.
Such a synthetic tissue which is biologically organized has
not been provided. Thus, the synthetic tissue of this
embodiment according to the present invention is new also
in view of the structure. Further, the preferable embodiment
25 having a biological integration capability with the
surroundings provides a synthetic tissue which has not exist
conventionally on the point that the synthetic tissue can
form a part of an organism after implantation. The present
invention can provide a synthetic tissue which does not
30 include any cell, even a cell which has been frozen once
and died. The tissue is still unique on the point that it
has an affinity with the surrounding even in such a case.

                                                        In   one
embodiment, the synthetic tissue of the present invention
is different from conventional synthetic tissues in that
the former comprises a cell. Particularly, a high density

5   that the density of $5 \times 10^8/cm^3$ at maximum can be included is
important. The present invention is important on the point
that it is suitable for implanting cells rather than
implanting the tissue.

10   Preferably, a synthetic tissue of the present
invention substantially comprise cells or a material derived
from the cells. Since the synthetic tissue is composed
substantially of only cells and a cell-derived material (e.g.,
extracellular matrix, etc.), the synthetic tissue can have

15   an increased level of biocompatibility and affinity. As used
herein, the terms "substantially comprise ...",
"substantially made of ...", and "substantially contain ..."
mean that cells and substances derived from the cells are
included, and also any other substance may be included as

20   long as it does not cause any harmful effect (herein, mainly,
bad effect on implantation), and should understood as such
herein. Such substances which do not cause any harmful effect
are known to those skilled in the art or can be confirmed
by conducting an easy test. Typically, such substances are,

25   but not limited to, any additives permitted by the Health,
Labor and Welfare Ministry, Food and Drug Administration
(FDA) or the like, ingredients involved in cell culture,
and the like. The cell-derived material representatively
includes extracellular matrices. Particularly, the

30   synthetic tissue or complex of the present invention
preferably comprises a cell and an extracellular matrix at
an appropriate ratio thereof. Such an appropriate ratio of
a cell and an extracellular matrix is from about 1:3 to about

- 114 -

20:1. The strength of the tissue is adjusted by the ratio
between a cell and an extracellular matrix. The ratio between
a cell and an extracellular matrix is adjusted for use in
accordance with application of cell implantation and physical
environment at the implantation site. Preferable ratio
varies depending on the treatment to be aimed. Such a
variation is apparent to those skilled in the art and can
be estimated by investigating the ratio of a cell in an organ
which is a target and an extracellular matrix.

Preferablly, a synthetic tissue substantially
comprising cells and an extracellular matrix derived from
the cells has not been known. Therefore, the present
invention provides a totally new synthetic tissue.

Preferably, an extracellular matrix which forms the
present invention includes, collagen I, collagen III,
vitronectin, fibronectin, and the like. It is preferable
that a variety of extracellular matrix includes all the listed
ingredients, and that they are integrated and mixed.
Alternatively, it is preferable that extracellular matrix
is dispersed across the entire body. Such a distribution
has a significant effect on the point that compatibility
and affinity with the environment can be improved when
implanted. The present invention is known to be
characterized in that adhesion to intercellular matrix which
promotes cell adhesion to a matrix, cell extension, and cell
chemotaxis is also promoted by including collagen (Types
I, III), vitronectin, fibronectin, and the like. However,
a synthetic tissue which includes collagen (Types I, III),
vitronectin, fibronectin, and the like has not been provided.
It is not intended to be constrained by the theory, but,
collagen (Types I, III), vitronectin, fibronectin, and the

like are contemplated to have a function in exercising the
biological integration capability with the surrounding.
Therefore, in the preferable embodiment, it is advantageous
that vitronectin are positioned to be dispersed on a surface
5   of the synthetic tissue or complex of the present invention.
It is considered that adhesion, affinity, and stability after
implantation are significantly different.

It is preferable that the fibronectin is also
10   positioned in the synthetic tissue or complex of the present
invention. It is known that fibronectin has a function in
cell adhesion, control of a shape of a cell, and adjustment
in cell migration. A synthetic tissue in which fibronectin
is expresse has not been provided. It is not intended to
15   be contrained by the theory, fibronectin is also
contemplated to have a function in exercising the biological
integration capability with the surrounding. Therefore, in
the preferable embodiment, it is advantageous that
fibronectin are also positioned to be dispersed on a surface
20   of the synthetic tissue or complex of the present invention.
It is considered that adhesion, affinity, and stability after
implantation are significantly different.

In the preferred embodiment, it is understood that
25   to position extracellular matrix used in the present
invention on the synthetic tissue or complex can be readily
achieved by the synthetic tissue production method of the
present invention. It is also understood that the production
method is not limited to this.
30

In more preferable embodiment, it is advantageous
to position the extracellular matrix used in the present
invention to be dispersed. Positioning extracellular

matrix into such a dispered state was impossible in convetional synthetic tissues. It is understood the present invention is the fist to provide such a tissue.

5   In the preferred embodiment, regarding extracellular matrix positioned to be dispersed on the synthetic tissue or complex, when distribution densities in any two section of 1 cm^2 are compared, the ratio is preferably within the range of about 1:3 to 3:1. Measurement of 10   distribution densities can be performed by any method known in the field of the art, for example, immune stainintg or the like.

In the preferred embodiment, regarding 15   extracellular matrix used in the present invention, when distribution densities in any two section of 1 cm^2 are compared, the ratio is preferably within the range of about 1:2 to 2:1, and further preferably, about 1.5:1 to 1.5:1. It is advantageous that extracellular matrix is uniformly 20   dispersed. Preferably, extracelluar matrix is dispersed substantially uniform, but it is not limited to this.

In one embodiment, extracellular matrix positioned in the present ivnetion may include collagen I, collagen 25   III, vitronectin, fibronectin or the like.

In an alternative embodiment, the synthetic tissue or complex of the present invention may employ heterologous cells, allogenic cells, isogenic cells or autologous cells. 30   In the present invention, it is found that even allogenic cells, particularly, mesenchymal cells are used, no adverse reactions, such as immune rejection reactions, is generated. Thus, the present invention ends to the development of the

treatment of ex vivo, and also a therapy which produces a
synthetic tissue using cells of others and utilize the tissue
without using an immuno rejection suppressor or the like.

5   In one preferred embodiment, the cells included in
the synthetic tissue or complex of the present invention
may be stem cells, differentiation cells, or they may include
both. In the preferred embodiment, the cells included the
three directional structure are mesenchymal cells. It is
10   not intended to restrained to the theory, the mesenchymal
cells are preferably used because the mesenchymal cells are
highly compatible with various organs such as heart, and
may have capability to differentiate into various organs
such as a heart.

15
          Such mesenchymal cells may be mesenchymal stem cells,
or may be mesenchymal differentiation cells.

          Examples of the mesenchymal cells used in the present
20   invention include, but not limited to, bone marrow cells,
adipocyte, synovial cell, myoblast, skeletal muscle cells,
and the like. Examples of mesenchymal cells as used herein
include stem cells derived from an adipose tissue, stem cells
derived from a bone marrow, and the like.

25
          In the preferred embodiment, it is advantageous that
the cells used in the present invention are cells derived
from the subject to which the synthetic tissue or complex
is applied. In such a case, cells as used herein also referred
30   to as autologous cells. By using autologous cells, immune
rejection reactions can be prevented or reduced.

          Alternatively, in another embodiment, the cells as

- 118 -

used herein may not be cells derived from a subject to which
the synthetic tissue or complex is applied. In such a case,
it is preferable that measures are taken to prevent immune
rejection reactions.

5

The synthetic tissue or complex of the present
invention may be provided as a drug. Alternatively, the
synthetic tissue or complex may be prepared by a physician
for therapy, or, a physician may first prepare the cells,
10   and then the third party may culture the cells and prepare
as a third-dimension structure for use in a surgery. In such
a case, culturing cells is not necessarily performed by a
physician, but can be performed by those skilled in the art
of cell culture. Those skilled in the art can determine
15   culturing conditions in accordance with a variety of the
cells and an implantation site to be targeted after reading
the disclosure herein.

In another embodiment, the synthetic tissue or
20   complex of the present invention is preferably isolated.
In this case, the term "isolate" means that the synthetic
tissue is detached from a scaffold, a support, and a culture
medium used in culture. If a synthetic tissue of the present
invention is substantially free of materials, such as a
25   scaffold and the like, it is possible to suppress adverse
reactions after implantation, such as immune rejection
reactions, inflammation reactions, and the like.

30   The base area of the synthetic tissue according to
the present invention may be, for example, 1 cm^2 to 20 cm^2.
However, the area is not limited to this range and may be
smaller than 1cm^2, or greater than 20cm^2. It is understood

- 119 -

that the essential feature of the present invention is that
a tissue of any size (area, volume) can be produced, and
it is not limited in the size.

5   In a preferable embodiment, the synthetic tissue of
the present invention is thick. The term "thick" in relation
to a synthetic tissue typically means that the synthetic
tissue has a thickness which provides a strength sufficient
to cover a site to which the synthetic tissue is implanted.

10   Such a thickness is, for example, at least about 50 μm, more
preferably at least about 100 μm, at least about 200 μm, at
least about 300 μm, even more preferably at least about 400 μm,
still more preferably at least about 500 μm, and still even
more preferably about 1 mm. It is recognized that, in some

15   cases, a tissue having a thickness of 3 mm or greater and
a tissue having a thickness of 5 mm or greater can be produced.
Alternatively, such a thickness may be, 1 mm or less. It
is understood that an essential feature of the present
invention is that a tissue or a complex having any thickness

20   can produced, and the tissue or complex is not limited in
the size.

          The present invention provides a scaffold-free
synthetic tissue or complex. By providing such a

25   scaffold-free synthetic tissue, a therapeutic method and
a therapeutic agent for providing an excellent condition
after implantation can be obtained.

          The scaffold-free synthetic tissue of the present

30   invention solves a long outstanding problem with biological
formulations, which is attributed to contamination of the
scaffold itself. Despite the absence of a scaffold, the
therapeutic effect is comparable with or more satisfactory

than conventional techniques.

In addition, when a scaffold is used, the alignment
of implanted cells in the scaffold, the cell-to-cell
5   adhesion, the in vivo alteration of the scaffold itself
(eliciting inflammation), the acceptance of the scaffold
to recipient tissue, and the like become problematic. These
problems can be solved by the present invention.

10   The synthetic tissue and the complex of the present
invention are also self-organized, and have biological
integration inside thereof. Also in this point, the present
invention is distinguished from conventional cell therapies.

15   The synthetic tissue and the complex of the present
invention are easy to form a three-dimensional structure,
and is thus easy to be designed into a desired form. The
versatility of the synthetic tissue and the complex of the
present invention should be noted.
20

The synthetic tissue and the complex of the present
invention have biological integration with recipient
tissues, such as surrounding tissues, cells, and the like.
Therefore, the post-operational acceptance is satisfactory,
25   and cells are reliably supplied to a local site, for example.
An effect of the present invention is that the satisfactory
biological integration capability allows the formation of
a tissue complex with another synthetic tissue or the like,
resulting in a more complex therapy.
30

Another effect of the present invention is that
differentiation can be induced after the synthetic tissue
or the complex is provided. Alternatively, differentiation

is induced before providing a synthetic tissue and/or a
complex, and thereafter, the synthetic tissue and/or the
complex are formed.

5   Another effect of the present invention is that the
cell implantation of the present invention provides a
satisfactory replacement and a comprehensive supply of cells
for covering an implanted site, compared to conventional
cell-only implantation and sheet implantation.

10
           The present invention provides an implantable
synthetic tissue having biological integration capability.
The above-described features and effects of the present
invention become it possible to treat a site which cannot
15   be considered as an implantation site for conventional
synthetic products. The present invention makes it possible
to provide a synthetic tissue or a three-dimensional
structure. The synthetic tissue of the present invention
has biological integration and actually works in implantation
20   therapies. The synthetic tissue is first provided by the
present invention, but is not provided by conventional
techniques.

           In addition, the present invention provides medical
25   treatment which provides a therapeutic effect by filling,
replacing, and/or covering an affected portion.

           In addition, when the synthetic tissue of the present
invention is used in combination with another synthetic
30   tissue (e.g., an artificial bone made of hydroxyapatite,
a microfibrous collagen medical device, etc.), the synthetic
tissue of the present invention is biologically integrated
with the other synthetic tissue, so that the acceptance of

the synthetic tissue can be improved to an extent which is not conventionally expected.

An extracellular matrix or a cell adhesion molecule, such as fibronectin, vitronectin, or the like, is distributed throughout the synthetic tissue of the present invention. In cell sheet engineering, a cell adhesion molecule is localized on a surface of culture cells which is attached to a culture dish. In the sheet of the cell sheet engineering, the cells are major components of the sheet. The sheet is nearly a mass of cells, on the bottom surface of which an adhesion molecule (glue) is added. On the other hadn, the synthetic tissue of the present invention is a real "tissue" such that an extracellular matrix covers cells. Thus, the present invention is significantly distinguished from conventional techniques.

A cell implanting method without a scaffold has been reported by Kushida A., Yamato M., Konno C., Kikuchi A., Sakurai Y., Okano T., J. Biomed. Mater. Res., 45:355-362, 1999, in which a cell sheet is produced using a temperature sensitive culture dish. Such a cell sheet engineering technique is internationally appraised due to its originality. However, a single sheet obtained by this technique is fragile. In order to obtain the strength that can withstand surgical manipulation, such as implantation, a plurality of sheets need to be assembled, for example. Such a problem is solved by the present invention.

A cell/matrix complex developed by the present invention does not require a temperature sensitive culture dish unlike the cell sheet technique. The cell/matrix complex is easy to form into a contractile three-dimensional

tissue. There is no technique in the world other than the
present invention, which can produce a contractile
three-dimensional complex having 10 or more layers without
using so-called feeder cells, such as rodent stroma cells,
5 at about three weeks. By adjusting conditions for matrix
production of the cell, it is possible to produce a complex
having a strength which allows surgical manipulation, such
as holding or transferring the complex, without a special
instrument. Therefore, the present invention is an original,
10 epoch-making technique in the world for reliably and safely
performing cell implantation.

In another embodiment, the synthetic tissue or
complex of the present invention is flexible. Due to the
15 flexibility, the synthetic tissue is particularly suitable
for reinforcement of motile organs. Examples of motile
organs include, but are not limited to, hearts, blood vessels,
muscles, and the like.

20 In another embodiment, the synthetic tissue or
complex of the present invention has dilation/contraction
ability. Due to the dilation/contraction ability, the
synthetic tissue is suitable for organs which expand and
contract, including, for example, hearts, muscles, and the
25 like. The dilation/contraction ability cannot be achieved
by cell sheet or the like prepared by conventional methods.
Preferably, a synthetic tissue of the present invention has
a sufficient strength to withstand the pulsation motion of
a heart. The strength sufficient to withstand pulsation
30 motion is, but is not limited to, at least about 50% of the
strength of naturally-occurring myocardium, preferably at
least about 75%, and more preferably at least about 100%.

- 124 -

In a preferable embodiment, the synthetic tissue or complex of the present invention has biological integration in all three dimensions. There are some synthetic tissues prepared by conventional methods, which have biological integration in two dimensions to some degree. However, no tissue having biological integration in all three dimensions can be prepared by conventional methods. Therefore, since the synthetic tissue of the present invention has biological integration in all three dimensions, the synthetic tissue is substantially implantable in any application.

Examples of biological integration which is an indicator of a synthetic tissue or complex of the present invention, include, but are not limited to, interconnection of extracellular matrices, electrical integration, the presence of intracellular signal transduction, and the like. The interaction of extracellular matrices can be observed with a microscope by staining intracellular adhesion as appropriate. Electrical integration can be observed by measuring electric potential.

In a preferable embodiment, the synthetic tissue of the present invention has a sufficient tissue strength for clinical applications. The sufficient tissue strength for clinical applications varies depending on a site to which the synthetic tissue is applied. Such a strength can be determined by those skilled in the art with reference to the disclosure of the specification and techniques well known in the art. The tensile strength of the synthetic tissue of the present invention may be low. The tensile strength becomes higher when the matrix concentration is increased, and becomes lower when the cell ratio is increased. The present invention is characterized in that the strength can

be adjusted as necessary. The present invention is also
characterized in that the strength can approximate to be
high or low relative to that of a tissue to be implanted.
Therefore, it is recognized that the goal can be set to comply
5   with any site.

In another embodiment, it is preferable that a
strength of the synthetic tissue or complex is sufficient
for having a self-supporting ability. Conventional
10 synthetic tissues do not have a self-supporting ability after
production. Therefore, when conventional synthetic tissues
are transferred, at least a part of them are injured.
However, when the technique of the present invention is used,
the synthetic tissue having the self-supporting ability is
15 provided. This means that the present invention provides
the synthetic tissue which cannot be provided by conventional
techniques. Preferable self-supporting ability is such
that, when a tissue is picked up with a tweezers having tips
of 0.5 to 3 mm (preferably, tips of 1 to 2 mm, and more
20 preferably, tips of 1 mm), the tissue is not substantially
destroyed. Herein, whether the tissue is not substantially
destroyed can be confirmed with eyes, but can be confirmed
by performing, for example, a water leakage test after the
tissue is picked up in the above-described conditions and
25 confirming that water does not leak. Alternatively, the
self-supporting ability as described above can also be
confirmed by not being destroyed when picked up by fingers,
instead of tweezers.

30 In a particular embodiment of the present invention,
the above-described clinical application is intended to a
bone, a joint, a cartilage, a meniscus, a tendon, a ligament,
a kidney, a liver, a synovial membrane, a heart, and the

like. The origin of cells contained in the synthetic tissue
of the present invention is not affected by clinical
applications.

5   Also, when a synthetic tissue of the present invention
is applied to a cartilage, the attachment ability of the
synthetic tissue can be tested by determining whether or
not the synthetic tissue remains attached without an
additional fixation procedure when the synthetic tissue is
10   implanted into an injured portion of the intra-articular
tissue (e.g., 2, 3 minutes after).

         In another aspect, the present invention provides
a cell culture composition for producing synthetic tissue
15   from a cell. The cell culture composition contains an
ingredient (e.g., commercially available medium, etc.) for
maintaining or growing the cell, and an ECM synthesis
promoting agent. The ECM synthesis promoting agent has been
described in detail in the above description of the synthetic
20   tissue production method. Therefore, the ECM synthesis
promoting agent includes ascorbic acid or a derivative
thereof (e.g., TGF-β1, TGF-β3, ascorbic acid 1-phosphate or
a salt thereof, ascorbic acid 2-phosphate or a salt thereof,
L-ascorbic acid or a salt thereof, etc.). The culture
25   composition of the present invention contains ascorbic acid
2-phosphate or a salt thereof at a concentration of at least
0.1 mM. Alternatively, in the case of a condensed culture
composition, the condensed culture composition contains
ascorbic acid 2-phosphate or a salt thereof at a concentration
30   which becomes at least 0.1 mM after preparation. Ascorbic
acid 2-phosphate or a salt thereof contained in the culture
composition of the present invention is present at a
concentration of at least 0.1 mM. When the culture

- 127 -

composition of the present invention is condensed, ascorbic
acid 2-phosphate or a salt thereof contained therein is
present at a concentration of at least 0.1 mM after
fomulation. It seems that 0.1 mM or more ascorbic acids have
5   substantially a constant effect. Thus, 0.1 mM can be said
to be sufficient. For TGF-β1 and TGF-β3, 1 ng/ml or more,
representatively 10 ng/ml, may be sufficient.

      Alternatively, the present invention may provide a
10   composition for producing a synthetic tissue, comprising
such an ECM synthesis promoting agent.

      In another embodiment of the present invention, an
ECM synthesis promoting agent used in the synthetic tissue
15   production method of the present invention includes ascorbic
acid 2-phosphate (Hata R., Senoo H., J. Cell Physiol., 1989,
138(1):8-16). In the present invention, by adding an at least
predetermined amount of ascorbic acid 2-phosphate, the
production of an extracellular matrix is promoted. As a
20   result, the resultant synthetic tissue or complex is made
rigid, and therefore, becomes easy to be detached.
Thereafter, the tissue undergoes self-contraction in
response to a stimulus of detachment. Hata et al. does not
disclose that the culture in medium supplemented with
25   ascorbic acid causes the tissue to be rigid and thus confers
to the tissue a property of being easily detached. Though
not wishing to be bound by any theory, a significant difference
between the present invention and Hata et al. is present
in cell density. Also, Hata et al. does not suggest the effect
30   of facilitating detachment of cells from a container for
culture. The present invention is the first to find the
effect of tissue contraction on development of
three-dimensional synthetic tissue from monolayer cultured

- 128 -

cells. The synthetic tissue of the present invention can be absolutely distinguished from conventional synthetic tissues, since the synthetic tissue of the present invention is produced via the procedures of tissue detachment and subsequent tissue contraction.

In a preferable embodiment, ascorbic acid 2-phosphate used in the present invention is typically present at a concentration of at least 0.01 mM, preferably at least 0.05 mM, more preferably at least 0.1 mM, even more preferably at least 0.2 mM, and still more preferably at least 0.5 mM, and still even more preferably 1.0 mM.

In one embodiment of the present invention, the cell density is, but is not particularly limited to, 5×10^4 to 5×10^6 cells per 1 cm^2. These conditions may be, for example, applied to myoblast. In this case, preferably, the ECM synthesis promoting agent may be ascorbic acids and may be provided at a concentration of at least 0.1 mM. This is because a thick synthetic tissue can be produced. In this case, if the concentration is increased, a synthetic tissue having a dense extracellular matrix is produced. If the concentration is low, the amount of an extracellular matrix is decreased but the self-supporting ability is maintained.

(Synthetic tissue for replacement and coverage)
In another aspect, the present invention provides a synthetic tissue or complex for reinforcement of a portion of an animal organism. The synthetic tissue or complex capable of such reinforcement is a technique achieved only after the synthetic tissue production method of the present invention is provided. Since the synthetic tissue or complex of the present invention has self-supporting ability, it

can be used in applications which are not conventionally
provided (e.g., filling (replacement) reinforcement, whole
reinforcement, no-leakage reinforcement, coverage, etc.).
The present invention has a significant effect such that
the filling and replacement reinforcement (i.e., cell supply)
was significantly improved. The present invention also
allows differentiation induction, which enlarges the range
of application of the present invention.

In a specific embodiment of the present invention,
the above-described reinforcement may be achieved by
disposing a synthetic tissue of the present invention to
cover the above-described portion. It is not possible to
use a synthetic tissue provided by conventional methods to
perform treatment by covering the above-described portion
(i.e., replacement and/or coverage application). Thus, the
synthetic tissue of the present invention can provide
applications which cannot be achieved by conventional
techniques.

Therefore, in the above-described specific
embodiment, the synthetic tissue or complex of the present
invention is resistant to dilation/contraction of the
above-described portion.

In a preferable embodiment, the synthetic tissue or
complex of the present invention advantageously has
biological integration.

In another preferable embodiment, the biological
integration includes at least one of interconnection of
extracellular matrices, electrical integration, and
intracellular signal transduction.

- 130 -

In another preferable embodiment, the synthetic
tissue or complex for reinforcement of the present invention
is formed by culturing a cell in the presence of an ECM
5   synthesis promoting agent.

In another embodiment, the synthetic tissue or
complex for reinforcement of the present invention comprises
a cell (autologous cell) derived from an animal to be treated
10   (e.g., a human). More preferably, a synthetic tissue for
reinforcement of the present invention comprises only a
cell(s) (autologous cell) derived from an animal to be treated
(e.g., a human) as a cell source.

15   Applications for the therapy utilizing the present
invention include, for example: cartilage full thickness
injury, cartilage partial injury; osteochondral injury;
osteonecrosis; osteoarthritis; meniscus injury; ligament
injury (chronic injury, degenerative tear, biological
20   augmentation for reconstruction surgery, etc.); rotator cuff
(particularly, chronic injury, degenerative tear, etc.);
delayed   union;   nonunion;   skeletal   muscle
repair/regeneration; cardiac muscle repair; (augmenting the
repair of necrotic tissue by ischemic-heart disease) or the
25   like.

(Therapy using replacement and coverage)
In another aspect, the present invention provides
a method for reinforcement of a portion of an animal organism.
30   The method comprises the steps of: A) disposing a synthetic
tissue or complex to replace or cover the portion; and
B) holding the synthetic tissue or complex for a time
sufficient to connect to the portion. Herein, to position

a portion for replacement typically means to perform
debridement or curettage of an affected portion as necessary,
to position the synthetic tissue or complex of the present
invention on the lesion, and to allow it to stand so as to
5   promote replacement. An objective of such replacement is
to fill cells. Techniques known in the art can be combined
and used. The step of disposing the synthetic tissue to cover
the portion can be carried out using a technique well known
in the art. The sufficient time varies depending on a
10   combination of the portion and the synthetic tissue, and
can be easily determined as appropriate by those skilled
in the art depending on the combination. Examples of such
a time include, but are not limited to, 1 week, 2 weeks,
1 month, 2 months, 3 months, 6 months, 1 year, and the like.
15   In the present invention, a synthetic tissue preferably
comprises substantially only cell(s) and material(s) derived
from the cell. Therefore, there is no particular material
which needs to be extracted after operation. The lower limit
of the sufficient time is not particularly important. In
20   this case, it can be said that the longer the time, the more
preferable the synthetic tissue. If the time is sufficiently
extremely long, it can be said that reinforcement is
substantially completed. Therefore, the time is not
particularly limited. The synthetic tissue of the present
25   invention is also characterized in that it is easily handled,
is not destroyed during an actual treatment, and facilitates
a surgery due to its self-supporting ability.

       In another embodiment, in a reinforcement method of
30   the present invention, the above-described portion
preferably includes bag-shaped organs (e.g., hearts, livers,
kidneys, etc.). In order to reinforce such a bag-shaped
tissue, it is necessary to replace or cover the organ. A

synthetic tissue resistant to applications for replacement
or covering is first provided by the present invention.
Therefore, the reinforcement method of the present invention
is advantageous over conventional techniques.

Alternatively, the above-described portion may
include a bone or cartilage. Examples of such portions
include, but not limited to, meniscus, ligament, tendon,
and the like. By the method of the present invention a
disease, injury, or condition of a heart, bone, cartilage,
ligament, tendon, or meniscus may be treated, prevented or
reinforced.

Particularly, in the reinforcement method of the
present invention, a synthetic tissue or complex of the
present invention is resistant to dilation/contraction of
the above-described portion. Examples of such
dilation/contraction include, but are not limited to, the
pulsation motion of a heart, the contraction of a muscle,
and the like.

In another preferable embodiment, in the
reinforcement method of the present invention, a synthetic
tissue or complex of the present invention has biological
integration (e.g., interconnection of extracellular
matrices, electrical integration, intracellular signal
transduction, etc.). The biological integration is
preferably provided in all three dimensions.

In another preferable embodiment, the reinforcement
method of the present invention further comprises culturing
a cell in the presence of an ECM synthesis promoting agent
to form a synthetic tissue or complex of the present invention.

An implantation/regeneration technique using the method
which comprises the step of culturing a cell in the presence
of an ECM synthesis promoting agent cannot be provided by
conventional techniques. The method provides a therapy for
5   diseases (e.g., cartilage injury, intractable bone fracture,
etc.), which cannot be achieved by conventional therapies.

In a preferable embodiment, in the reinforcement
method of the present invention, the cell used in the synthetic
10   tissue or complex of the present invention is derived from
an animal to which the synthetic tissue is to be implanted
(i.e., an autologous cell). By using an autologous cell,
adverse side effects, such as immune rejection reactions
or the like, can be avoided.

15

In another preferable embodiment, the portion is a
heart.

Applications for the therapy utilizing the present
20   invention include, for example: cartilage full thickness
injury, cartilage partial injury; osteochondral injury;
osteonecrosis; osteoarthritis; meniscus injury; ligament
injury (chronic injury, degenerative tear, biological
augmentation for reconstruction surgery, etc.); rotator cuff
25   (particularly, chronic injury, degenerative tear, etc.);
delayed   union;   nonunion;   skeletal   muscle
repair/regeneration; cardiac muscle repair; (augmenting the
repair of necrotic tissue by ischemic-heart disease) or the
like.

30

For some organs, it is said that it is difficult to
radically treat a specific disease, disorder, or condition
thereof (e.g., refractory heart diseases). However, the

present invention provides the above-described effect,
thereby making possible a treatment which cannot be achieved
by conventional techniques. It has been clarified that the
present invention can be applied to radical therapy.
Therefore, the present invention has usefulness which cannot
be achieved by conventional medicaments.

Thus, the present invention provides a method for
treating a portion of an organism of an animal, comprising:
A) positioning the synthetic tissue or complex so as to cover
the portion; and B) retaining the synthetic tissue for a
time period which is sufficient for the condition of the
portion of the organism to be improved. Such an improvement
in the condition can be determined can be determined in
accordance with the function of the portion to be treated.
For example, when a heart should be treated, an improvement
in the condition can be determined by checking a cardiac
function (heartbeat, bloodstream, or the like). If a bone
should be treated, an improvement in the condition can be
determined by observing osteogensis by using roentgen, CT
scan, or the like. In the case of a bone, an improvement
in the condition can be determined by measuring its strength
or by evaluating bone marrow and/or a bone substance by using
MRI. If a cartilage or meniscus should be treated, a surface
of a joint can be observed by an arthroscopy. Further, it
is possible to determine an improvement in the condition
by performing a biomechanical inspection under arthroscopy.
It is also possible to determine an improvement in the
condition by confirming a repairing condition by using MRI.
Regarding ligament, it is possible to determine by confirming
whether there is laxity by a joint stability inspection.
Further, an improvement of the condition can be determined
by confirming a continuousness of a tissue by an MRI. In

the case of any tissue, it is possible to determine whether
the condition is improved by performing a biopsy of the tissue
and making a histological evaluation.

In a preferred embodiment the treatment treats,
prevents, prognosis, or enhances a disease, injury, or
condition of a heart, bone, cartilage, ligament, tendon,
or meniscus. Preferably, the synthetic tissue or the complex
has a self-supporting ability. For such a synthetic tissue,
those skilled in the art can use a synthetic tissue of any
form described above herein, and a variant thereof.

(Combined therapy)

In another aspect, the present invention provides
a regeneration therapy which uses a cytokine, such as BMP
(e.g., BMP-2, BMP-4, BMP-7, etc.), TGF-β1, TGF-β3, HGF, FGF,
IGF, or the like, in combination with a synthetic tissue.

Some cytokines used in the present invention are
already commercially available (e.g., BMP (Yamanouchi
Pharmaceutical), bFGF2 (Kaken Pharmaceutical), TGF-β1 (for
research only, HGF-101 from Toyo Boseki, etc.). However,
these cytokines can be prepared by various methods and can
be used in the present invention if they are purified to
an extent which allows them to be used as a medicament. A
certain cytokine can be obtained as follows: primary cultured
cells or an established cell line capable of producing the
cytokine is cultured; and the cytokine is separated from
the culture supernatant or the like, followed by
purification. Alternatively, a gene encoding the cytokine
is incorporated into an appropriate vector by a genetic
engineering technique; the vector is inserted into an
appropriate host to transform the host; a recombinant

- 136 -

cytokine of interest can be obtained from the supernatant
of the transformed host culture (e.g., Nature, 342,
440(1989); Japanese Laid-Open Publication No. 5-111383;
Biochem-Biophys. Res. Commun., 163, 967 (1989), etc.). The
5   above-described host cell is not particularly limited and
can be various host cells conventionally used in genetic
engineering techniques, including, for example, *Escherichia
coli*, yeast, animal cells, and the like. The thus-obtained
cytokine may have one or more amino acid substitutions,
10   deletions and/or additions in the amino acid sequence as
long as it has substantially the same action as that of the
naturally-occurring cytokine. Examples of a method for
introducing the cytokine into patients in the present
invention include, but are not limited to, a Sendai virus
15   (HVJ) liposome method with high safety and efficiency
(Molecular Medicine, 30, 1440-1448(1993); Jikken Igaku
(Experimental Medicine), 12, 1822-1826 (1994)), an
electrical gene introduction method, a shotgun gene
introduction method, a ultrasonic gene introduction method,
20   and the like. In another preferable embodiment, the
above-described cytokines can be administered in the form
of proteins.

(Production method of synthetic tissue having
25   desired thickness)
Another aspect of the present invention provides a
method for producing a synthetic tissue or complex having
a desired thickness. This method comprises: A) providing
cells; B) positioning the cells in a container having the
30   base area sufficient for accommodating the synthetic tissue
or complex having the desired size, which contains an ECM
synthesis promoting agent (e.g., ascorbic acids, TGF-β1,
TGF-β3, etc.); C) culturing the cells in the container with

a cell culture medium including the ECM synthesis promoting
agent for a time sufficient for forming the synthetic tissue
or complex having the desired size to convert the cells into
a synthetic tissue; and D) adjusting the thickness of the
5   synthetic tissue to obtain a desired thickness by a physical
stimulation or a chemical stimulation. Herein, the steps
of providing the cells, positioning the cells, stimulating
and converting into the tissue or complex are described with
respect to the production method for the synthetic tissue
10   or complex of the present invention in detail, and it is
understood that any embodiment can be employed.

       Next, examples of the physical or chemical
stimulation to be used may include, but not limited to, use
15   pipetting, use of actin interacting substance. Pipetting
may be preferable because operation is easy and no harmful
substance is produced. Alternatively, examples of the
chemical stimulation to be used may include actin
depolymerizing factors and actin polymerizing factor.
20   Examples of such an actin depolymerizing factor may include
ADF (actin depolymerizing factor), destrin, depactin,
actophorin, cytochalasin, NGF (nerve growth factor) and the
like. Examples of the actin polymerizing factor include
LPA (lysophosphatidic acid), insulin, PDGFa, PDGFb,
25   chemokine, and TGFb. The polymerization or
depolymerization of actin can be observed by checking the
activity to actin. It is possible to test any substance
whether it has such an activity. It is understood that a
substance which is tested as such and identified can be used
30   for achieving the desired thickness in production of the
synthetic tissue of the present invention. For example, in
the present invention, the adjustment of the desired
thickness can be achieved by adjusting the ratio between

the actin depolymerizing factor and actin polymerizing factor.

(Composite tissue)

Another aspect of the present invention also provides a tissue complex including an implantable synthetic tissue and another synthetic tissue. Herein, another tissue may either be a synthetic tissue included within the scope of the present invention, or a synthetic tissue out of the scope (i.e., conventional tissues). Conventional tissues (e.g., an artificial bone, microfibrous collagen medical device, etc.,) do not have a biological integrating ability or have a biological integrating ability which cannot stand the practical use. Thus, it was almost impossible to form such a tissue complex. It is understood that, according to the present invention, a cartilage can be combined to a bone for treatment. For the case of a cavity in a bone or the like, particularly, for the case of treatment of bone cartilage complex, by using a tissue complex of an artificial bone (e.g., hydroxyapatite construct such as NEO BONE, a microfibrous collagen medical device, etc.) and the synthetic tissue or complex of the present invention, it is possible to treat the bone by the artificial bone, and the cartilage on the bone by the synthetic tissue at the same time. It is understood that the synthetic tissue or complex of the present invention is combined to an artificial bone and used for treatment. Herein, the implantable synthetic tissue or complex of the present invention substantially comprises, for example, cells and substances derived from the cells, and more preferably, cells and extracellular matrix derived from the cells. The extracellular matrix as used herein is selected from the group consisting of collagen I, collagen III, vitronectin, and fibronectin.

As used herein, the term "tissue complex" refers to
a tissue obtained by combining a synthetic tissue or complex
of the present invention with another synthetic tissue
5   (including a synthetic tissue or complex of the present
invention). Such a tissue complex can be used for a treatment
of a plurality of tissues. For example, such a tissue complex
can be used for treatment of both cartilage and bone.

10   In the case there is a large defect of soft tissue
(e.g., meniscus, etc.), the synthetic tissue of the present
invention can be coupled to another synthetic tissue
(microfibrous collagen medical device (e.g., CMI (Amgen,
USA), Integran® (Nippon Zoki Pharmaceutical), hyaluronic
15   acid gel, collagen gel, agarose gel, alginate gel, beads
etc.) to promote biological integration between another
synthetic tissue and an implantation cells.

Preferably, in the complex of the present invention,
20   an implantable synthetic tissue and another synthetic tissue
are biologically integrated. Such integration can be
produced by culturing two tissues in contact. Such a
biological integration is mediated by extracellular matrix.

25   Hereinafter, the present invention will be described
by way of examples. Examples described below are provided
only for illustrative purposes. Accordingly, the scope of
the present invention is not limited except as by the appended
claims.

30

(Examples)
In the examples below, animals were treated in

accordance with rules defined by Osaka University (Japan)
and were cared for in the spirit of animal protection.

(Example 1: Synovial cell)
In this example, various synovial cells were used
to produce a synthetic tissue as follows.

<Preparation of cells>
Synovial cells were collected from a knee joint of
a pig (LWD ternary hybrid, 2-3 months old upon removal of
cells), followed by treatment with collagenase. The cells
were cultured and subcultured in 10% FBS-DMEM medium (FBS
was obtained from HyClone, DMEM was obtained from GIBCO).
It has been reported that 10th passage synovial cells still
have pluripotency. Although cells of 10 or less passages
were used in this example, cells of more than 10 passages
may be used depending on the application.
Autotransplantation was performed for humans, where a
sufficient number of cells were used and the cells were
cultured for a short period of time so as to reduce the risk
of infection or the like.

Considering these points, cells of various passages
were used. Actually, primary culture cells, first passage
cells, second passage cells, third passage cells, fourth
passage cells, fifth passage cells, sixth passage cells,
eighth passage cells, and tenth passage cells were used in
experiments. These cells were used for synthetic tissues.

<Preparation of synthetic tissue>
Synovial cells (4.8×10^6) were cultured in 2 ml of
10% FBS-DMEM medium in a 35-mm dish, a 60-mm dish, or 100-mm
dish (BD Biosciences, culture dish and multiwell cell culture

plate). In this case, ascorbic acid was added. The dishes,
the ascorbic acid concentrations, and the cell concentration
are described below.

5   Dishes: BD Biosciences, cell culture dishes and multiwell
cell culture plates

Ascorbic acid 2-phosphate: 0 mM, 0.1 mM, 0.5 mM, 1 mM, 2 mM,
and 5 mM

10

The number of cells: 5×10^4 cells/cm^2, 1×10^5 cells/cm^2,
2.5×10^5 cells/cm^2, 4.0×10^5 cells/cm^2, 5×10^5 cells/cm^2,
7.5×10^5 cells/cm^2, 1×10^6 cells/cm^2, 5×10^6 cells/cm^2, and
1×10^7 cells/cm^2

15

        Medium was exchanged two times per week until the
end of a predetermined culture period. At the end of the
culture period, a cell sheet was detached from the dish by
pipetting circumferentially around the dish using a 100-μl
20   pipetteman. After detachment, the cell sheet was made as
flat as possible by lightly shaking the dish. Thereafter,
1 ml of medium was added to completely suspend the cell sheet.
The cell sheet was allowed to stand for two hours, resulting
in the contraction of the cell sheet into a three-dimensional
25   form. Thus, a synthetic tissue was obtained (Fig. 1).

        <Hematoxylin-Eosin (HE) Staining>
        The acceptance or vanishment of cells in a sheet was
observed by HE staining. The procedure is described as
30   follows. A sample is optionally deparaffinized (e.g., with
pure ethanol), followed by washing with water. The sample
is immersed in Omni's hematoxylin for 10 min. Thereafter,
the sample is washed with running water, followed by color

development with ammonia in water for 30 sec. Thereafter,
the sample is washed with running water for 5 min and is
stained with eosin hydrochloride solution for 2 min, followed
by dehydration, clearing, and mounting.

(Various extracellular matrix staining)

1. Make 5 μm thick sections from frozen block.

2. Sections are fixed in acetone at ~20°C for 9-10 mins.
(Paraffin   blocks   should   be   deparaffinized   and
rehydrated).

3. Endogenous peroxide activity is blocked in 0.3% H_2O_2 in
methanol for 20 mins at RT.

(1 ml 30% H_2O_2 + 99 ml methanol)

4. Wash with PBS (3 × 5 mins).

5. Incubate with primary monoclonal antibody (a mouse or
rabbit antibody against each extracellular matrix protein)
in a moist chamber at 4°C for overnight (1 μl antibody + 200 μl
PBS per slide).

6. Next day wash with PBS (3 × 5 mins).

7. Apply anti mouse and anti rabbit no. 1 Biotynalated link
for 30 mins ~1 hrs at RT.

(apply about 3 drops directly on slide).

8. Wash with PBS (3 × 5 mins).

9. Apply about 3 drops directly Streptavidin HRP no. 2 for
LSAB. 10-15 mins.

10. Wash with PBS (3 × 5 mins).

11. Apply DAB (5 ml DAB+5 μl H_2O_2).

12. Observe under microscope for brownish colour.

13. Dip in water for 5 mins.

14. Apply HE for 30 sec-1 min.

15. Wash several times.

16. Ion exchange water wash 1 time.

17. 80% ethanol wash for 1 min.

18. 90% ethanol wash for 1 min.
19. 100% ethanol wash for 1 min (3 times).
20. Xylene wash for 1 min (3 times), Coverslip.
21. Examine color development.

5

An exemplary result is shown in Figure 1. As shown
in the right portion of Figure 1, when ascorbic acid
2-phosphate was added as an ECM synthesis promoting agent,
a contractile three-dimensional tissue of the cells was only

10   slightly observed. On the other hand, by detaching the
sheet-like cells from the base of the culture dish and allowing
the cells to self organize, the cells were promoted to be
layered and were accelerated into a three-dimensional
structure, as shown in the left portion of Figure 1. As shown

15   in a left portion of Figure 1, large tissue without a hole
was also produced when synovial cells were used. This tissue
was thick and its extracellular matrix was rich as shown
in a right portion of Figure 1. When ascorbic acid
2-phosphate was added at a concentration of 0.1 mM or more,

20   the formation of an extracellular matrix was promoted
(Figure 2). Figure 3 shows an enlarged view of a synthetic
tissue on Day 3, 7, 14, and 21. As can be seen, after 3 days
of culture, the tissue was already so rigid that it can be
detached (Figure 3). As the number of culture days is

25   increased, the density of the extracellular matrix fluctuates
and increases.

The tissue was detached from the base of the culture
dish and self-contracted. The synthetic tissue was prepared
30   in a sheet form. When the sheet was detached from the dish
and was allowed to stand, the sheet self contracted into
a three-dimensional structure. It is seen that a number of
layers of cells exist in the tissue.

Next, various markers including extracellular matrix markers were stained.

Figure 4 shows the result of staining extracellular matrix. It can be seen that various extracellular matrix components (collagen I, II, III, fibronectin, vitronectin, etc.) existed. Immunostaining was conducted, so that collagen I and III were strongly stained while collagen II staining was limited to a portion. By being strongly magnified, it can be confirmed that collagen was stained at a site slightly away from the nuclei, i.e., collagen was a part of the extracellular matrix. On the other hand, fibronectin and vitronectin, which are believed to be important cell adhesion molecules. By being strongly magnified, it can be confirmed that fibronectin and vitronectin were stained at a region close to nulei unlike collagen, i.e., fibronectin and vitronectin existed around the cells.

These results demonstrated that cells of at least 3 to 8 passages are preferable for production of synthetic tissue.

For comparison, a normal tissue and a collagen sponge (CMI, Amgen, USA) were stained. Figure 5 shows the normal tissue (normal synovial membrane tissue, tendon tissue, cartilage tissue, skin, and meniscus tissue). Figure 6 shows the stained collagen sponge, which was the comparative example. From the left, fibronectin, vitronectin, negative control, and HE staining are indicated. As can be seen, the conventional synthetic tissue was not stained with fibronectin or vitronectin. Therefore, the synthetic

tissue of the present invention is different from
conventional synthetic tissues.   Existing collagen
scaffolds do not contain fibronectin and vitronectin
(adhesion agents).   In view of this, the originality of the
5   synthetic tissue of the present invention is clearly
understood.   No stain is found in the extracellular matrix.
When the synthetic tissue of this example was compared with
normal tissue, the synthetic tissue has a lower extracellular
matrix density and had a structure different from normal
10   tissue.

       Further, when the synthetic tissue of the present
invention was contacted with a filter paper in order to remove
moisture from the tissues, the filter is adhered to the
15   synthetic tissue, and it was difficult to manually detach
the synthetic tissue of the present invention.
          In order to determine the collagen concentration,
the collagen content was measured.   The result is shown in
Figures 7 and 8.   As can be seen, the amount of hydroxyproline
20   clearly indicates that when 0.1 mM or more ascorbic acid
2-phosphate was added, the production of collagen was
significantly promoted.   The amount of produced collagen is
substantially proportional to the time period of culture
(Figure 8).

25

          (Example 2: Measurement of collagen production)
          Next, it was determined whether or not collagen
(extracellular matrix) is sufficiently secreted after
implantation of a synthetic tissue of the present invention.
30   The following protocol was used.

          <Method>
          Culture periods: 3 days, 7 days, 14 days, and 21 days,

Concentrations of ascorbic acid 2-phosphate: 0 mM,
0.1 mM, 1 mM, and 5 mM

Under the above-described conditions, a synovial
membrane-derived synthetic tissue was produced.

6 N HCl was added to culture medium for the synthetic
tissue, followed by hydrolysis at 105°C for 16 hours. The
medium was oxidized with chloramine T. Thereafter, the
synthetic tissue was subjected to color development using
Ehrlich's   Reagent   Solution   (2 g   of
p-dimethylamino-benzaldehyde + 3 ml of 60% perchloric acid;
isopropanol was diluted at 3:13), followed by measurement
of absorbance.

<Results>

1) The quantities of collagen produced was dependent
on the ascorbic acid concentration in the following manner:
0 mM << 5 mM < 1 mM ≤ 0.1 mM (Figure **7 and 8**).

2) It was demonstrated that the quantity of produced
collagen is increased with an increase in the culture time
period.

(Example 3: Influences of the size of a dish, the
number of cells, and the number of passages)

Next, influences of the size of a dish and the number
of passages were investigated.

Figure **9** shows the formation of synthetic tissues
where the number of cells and the number of the passage were
changed. A synthetic tissue was formed in all concentrations
tested.

Under the conditions of the above-described
Example 1, a similar experiment was conducted where the sizes
of dishes were 35 mm, 65 mm, and 100 mm and the number of
passages were 5 to 7 (Figure 10).

5

The results are shown in Figures 9 and 10. Figure 9
shows the states of synthetic tissues, where the number of
passages was changed. Figure 10 shows the states of
synthetic tissues, where the size of a dish was changed.

10   As can be seen from the figures, it was demonstrated that
a synthetic tissue can be formed using any size of dish and
any number of passages.

15

As shown in Figure 9, basically, a greater number
of cells may be preferable for the purpose of matrix production.
However, when an excessive number of cells were provided,
the cells produced an excessive level of contraction force,

20   so that the cell sheet was detached on the day following
the start of culture. Therefore, it was demonstrated that
when a larger synthetic tissue is desired, it is preferable
to deassimate cells at a relatively small concentration.
Particularly, in order to control the strength or the like

25   of a synthetic tissue, a relatively small cell concentration
seems to be preferable. As can be seen from the figure, when
the number of passages was five, the resultant cell sheet
was spontaneously detached if the cell concentration was
$5.0 \times 10^5/cm^2$, and was not spontaneously detached if the cell

30   concentration was $2.5 \times 10^5/cm^2$. Also, when the number of
passages was six or more, the resultant cell sheet was
spontaneously detached if the cell concentration was
$7.5 \times 10^5/cm^2$, and was not spontaneously detached if the cell

concentration was $5.0 \times 10^5/cm^2$. Therefore, the production
of a preferable synthetic tissue of the present invention
seems to require a sufficient number of cells and a relatively
great number of passages. Fourth passage cells were used
5   to produce a trial synthetic tissue. It was spontaneously
detached when the cell concentration was $40 \times 10^5/cm^2$. Thus,
there seems to be a close relationship between the strength
of a synthetic tissue and the number of passages. Various
synthetic tissues can be produced, depending on the
10 application. According to these results, cells capable of
withstanding implantation seems to be obtained by culturing
fifth passage cells at a concentration of $4.0 \times 10^5/cm^2$, however,
the present invention seems not to be limited to this.

15   Similarly, the strength of tissues consisting of
other cells is demonstrated to be able to be regulated by
changing the cell concentration. Under the conditions
described in Example 1, myoblasts can be used to produce
a synthetic tissue and the influence of cell density on the
20 strength of the synthetic tissue can be measured. Under the
conditions described in Example 28, synovial cells can be
used to produce a synthetic tissue and the influence of cell
density on the strength of the synthetic tissue can be measured.
Under the conditions described in Example 12, fat-derived
25 cells can be used to produce a synthetic tissue and the
influence of cell density on the strength of the synthetic
tissue can be measured.

         (Example 4: Measurement of mechanical properties)
30   In this example, cells (4×10^5 cells/cm^2) were cultured
in medium containing ascorbic acid 2-phosphate for three
weeks. Following detachment at 48 hours, the mechanical
properties of the tissue were investigated. The protocol

will be described below.

The mechanical properties were examined by a tensile test.

Figures **11** and **12** show the outer appearance of a testing apparatus. Figure **11** shows a test piece holding portion (an original piece is shown). As shown in Figure **12**, the opposite ends of a synthetic tissue were held by the test piece holding portion. A marker was attached to the synthetic tissue for ease of measurement. Figure **13** shows the attachment of the marker. Figure **14** shows an enlarged view of the test piece holding portion. Figure **15** shows the state of the synthetic tissue after a tensile test.

A synthetic tissue was held as shown in the figures and a marker was attached to the synthetic tissue, followed by a tensile test. The maximum load was 1.89 N, and the Young's modulus was 19.2 Mega pascal. As a reference, the maximum load (tension) of cartilage is typically 0.7 and that of skin is 1.2. The Young's modulus of cartilage is 10 MPa and that of skin is 35 Mpa. Thus, it was demonstrated that the synthetic tissue of the present invention has substantially the same mechanical strength as that of skin, cartilage, or the like, and can resist surgical handling.

The results of the experiment are shown in Figures **16** and **17**. The results demonstrate that the maximum load was 1.89 N and 1.9 N, respectively. Young's modulus (tangent tensile modulus) was 19.2 MPa.

(Example 5: Determination of self-supporting ability)

Next, the self-supporting ability of a synthetic
tissue of the present invention was tested. The synthetic
tissue was held and tested using curved fine forceps A-11
(made of stainless steel; full length: 120 mm; curved: 20 mm,
tip: 0.1 mm; manufactured by Natsume Seisakusho). It was
determined by visual inspection whether or not the synthetic
tissue has self-supporting ability. If the synthetic tissue
was divided into a plurality of pieces, it was determined
to lacking self-supporting ability. The same result was
obtained when another forceps, e.g., curved fine forceps
A-12-2 (made of stainless steel, full length: 100 mm; tip:
0.05 mm; manufactured by Natsume Seisakusho) were used by
another experimenter performing the same expriment.

The self-supporting ability may be determined
immediately after detaching a synthetic tissue off or after
preserving a detached synthetic tissue.

None of the synthetic tissues comprising
cardiomyocytes, myoblasts, and synovial cells, which are
produced in the presence of a three-dimensional promoting
agent comprising ascorbic acid as described in the above
examples, had self-supporting ability. In contrast, it was
already difficult to hold a synthetic tissue produced in
the absence of such an agent with forceps upon detachment,
so that lack of self-supporting ability was confirmed.

Therefore, 1) if a sheet is easily detached by
circumferential pipetting; and 2) if the detached sheet is
easily attached to a target site by lightly touching an edge
thereof, the sheet spontaneously contracts to have sufficient
strength.

Therefore, self-supporting ability is a property
which was first obtained by the method of the present
invention.

5   (Example 6: Osteogenic differentiation induction)
          In this example, it was determined whether or not
the synthetic tissue of the present invention works when
osteogenesis was induced in the synthetic tissue.

10   It was confirmed that synovial cells can be cultured
in osteogenesis induction medium (10% FBS-DMEM+0.1 μM
dexamethasone, 10 mM beta glycerophosphate, 0.2 mM ascorbic
acid 2-phosphate) from the beginning to produce a synthetic
tissue.

15
          Also, it was confirmed that a synthetic tissue was
produced without osteogenesis induction, and thereafter,
the medium was exchanged with osteogenesis induction medium
and the tissue was cultured, so that calcificated bone was
20   generated in the synthetic tissue. The result is shown in
Figure 18.

          Whereas a synthetic tissue without differentiation
induction appears to be transparent, an ossificated synthetic
25   tissue has a white colour. The synthetic tissue was strongly
stained with Alizarin Red, and was also strongly stained
by alkali phosphatase (ALP) staining as compared to the
control. Thus, it was confirmed that the synthetic tissue
of synovial cells is capable of osteogenesis.

30
          (Example 7: chondrogenesis induction)

In this example, it was determined whether or not chondrogenesis induction can be used for the production method of the synthetic tissue of the present invention.

5   (Culture conditions)
          Cell density: 4×10^4 cells/cm^2
          Conditions: CO_2 5%, air 95%, 37°C

These conditions and a chondrogenesis induction
10   medium described below were used to produce a synthetic tissue.

Cartilage differentiation induction medium: DMEM (GIBCO), FBS (HyClone) 10%, ITS+Premix (insulin, transferrin,
15   selenious acid) (BD Biosciences) 6.25 µg/ml, dexamethasone (Sigma) 10^{-7} M, ascorbic acid (WAKO) 50 µg/ml, pyruvic acid (SIGMA) 100 µg/ml.

The results are shown in Figure 19. The cells were
20   induced into cartilage. From the left, a typical medium, a chondrogenesis induction medium, a chondrogenesis induction medium+BMP-2, and a chondrogenesis induction medium+TGF-b1 were used to culture a synthetic tissue. All of the tissues were stained blue with Alcian blue staining.
25   It was confirmed that a cartilage-like matrix production was accelerated. Such an effect is significant for cells cultured in medium containing BMP-2. The result of quantification of staining ability is shown in Figure 20.

30   Expression of cartilage-associated genes (aggrecan, Col II, Sox9) in the synthetic tissue is shown in Figure 21. When the synthetic tissue was transferred from the typical medium (leftmost column) to the chondrogenesis induction

medium (middle column), expression of the Sox9 gene, which
is a chondrogenesis marker, was increased. When the
synthetic tissue was further cultured in the chondrogenesis
induction medium+BMP-2, expression of the collagen II gene
5   was also increased. Thus, stronger chondrogenesis could be
confirmed. Figure 22 shows the results of comparison of a
chondrogenesis reaction between a monolayer culture synovial
cell and a synovial cell in a three-dimensional synthetic
tissue, when the same differentiation inducing stimulus was
10   applied. When counted from the left, odd-numbered columns
indicate monolayer culture, while even-numbered columns
indicate three-dimensional synthetic tissue, where culture
was performed under the same culture conditions. When the
chondrogenesis induction medium or the chondrogenesis
15   induction medium+BMP-2 was added as a stimulus, it was
confirmed that the chondrogenesis marker gene was
significantly expressed in the synthetic tissue. Thus, the
three-dimensional synthetic tissue was confirmed to have
strong chondrogenesis ability.
20

(Example 8: Repair of a pig cartilage)
Next, it was determined whether or not cartilage can
be repaired. An allogenic synthetic tissue was used.

25   To determine the presence or absence of the adhesion
capability of a synthetic tissue, an allogenic synthetic
tissue was implanted onto a pig cartilage piece. The
synthetic tissue was prepared under conditions where the
number of cells was 4.0×10^6 cells/35-mm dish, the
30   concentration of ascorbic acid was 1 mM, and the culture
period was 7 to 14 days. A wound having a diameter of 6 mm
was generated on the cartilage piece. An upper layer zone
thereof was cut off from the cartilage piece using a scalpel.

Chondroitinase ABC (1 U/ml) was added. The cartilage piece
was allowed to react for 5 minutes. A synthetic tissue was
sized to have a diameter of 6 mm and was implanted, followed
by culture for 7 days. The synthetic tissue is closely
5   attached to the attachment surface of the cartilage piece.
Fibronectin   aggregated   on   the   attachment   surface
(Figure 23).

        Next, pig cartilage implantation was performed. As
10   described above, a wound having a diameter of 6 mm was created
in a medial femoral condyle. An upper layer zone thereof
was cut off from the cartilage piece using a scalpel.
Chondroitinase ABC (1 U/ml) was added. The cartilage piece
was allowed to react for 5 minutes. A allogenic synthetic
15   tissue was sized to have a diameter of 6 mm and was implanted,
followed by culture for 7 days. The results are shown in
Figure 24. Figure 25 shows a strongly enlarged view of a
culture portion of a surface of the cartilage adhered to
the synthetic tissue of Figure 24. The left portion of
20   Figure 25 is a photograph showing the result of HE staining,
the middle portion is a photograph showing the result of
staining with anti-fibronectin antibodies, and the right
portion is a photograph showing the result of staining with
anti-vitronectin antibodies. As indicated by an arrow (the
25   interface between the synthetic tissue and the cartilage
tissue), it was demonstrated that the matrix of the synthetic
tissue was directly attached to the cartilage matrix, but
not via cells. It is shown that fibronectin and vitronectin
were accumulated at the adhesion surface. Thus, the results
30   suggest that these adhesion molecules are involved in
adhesion between a synthetic tissue and a recipient tissue.
Therefore, the present invention is also characterized in
that the synthetic tissue is more effectively adhered to

- 155 -

in vivo tissue than conventional synthetic tissues, or cells.

Further, the tissue was examined after one month of
implantation. The result is shown in Figure **26**. As can be
5   seen, it is confirmed that the synthetic tissue was
biologically integrated with the cartilage injury portion
and was accepted without inflammation. The surface layer
portion of the synthetic tissue was made mainly of
fibroblast-like cells as shown in Figure **27**. On the other
10   hand, a deeper layer portion of the synthetic tissue was
made mainly of cartilage-like cells as shown in Figure **28**.
Therefore, the implanted synthetic tissue had differentiated
into cartilage-like tissue over time. No significant
rejection was confirmed in any period of time, and rejection
15   which is expected for allogenic implantation, was not
observed.

Therefore, it was found that the allogenic synthetic
tissue can be implanted without a side effect.
20

(Example 9: Repair of a pig meniscus)
Next, it was determined whether or not the synthetic
tissue of the present invention is applicable to repair of
meniscus.
25

As in the above-described Example 6, an allogenic
synthetic tissue was prepared under conditions where the
number of cells was 4.0×10^6 cells/35-mm dish, the
concentration of ascorbic acid was 1 mM, and the culture
30   period of time was 7 to 14 days. A portion having a diameter
of 6.5 mm was removed from a meniscus (Figure **29**), and the
synthetic tissue was implanted thereinto. The portion
containing the implant was covered with a collagen sheet

(Nipro) for protection until the synthetic tissue was accepted (Figure 30). The pig was kept for one month. The protocol is described below.

5   (Anesthesia)

A pig 15 to 17 weeks old (LWD ternary hybrid) was intramuscularly injected via the dorsal portion of its neck with 20 mg/kg Ketaral + 10 mg/kg Sexactal. Thereafter, an infusion route was provided in the ear vein, and thereafter,

10   the respiratory tract was secured using endotracheal intubation. Diprivan was continuously administered at a rate of 0.5 mg/kg/hr to maintain anesthesia. An antibiotic (Cefamezin,   1 g)   was   administered   to   prevent post-operational infection.

15

        (Operation)

The animal was positioned and an operation portion was cleaned with a sterilized drape. A knee joint was accessed by medial para-patellar approach. After detecting

20   the internal articular capsule, the middle portion at the medial collateral ligament (MCL) of the knee was defected. A cylinder-shaped cavity (diameter: 6.5 mm) was created using the mosaic plasty DP (Smith & Nephew) (Figure 29). The cavity was filled with the synthetic tissue (Figure 30), followed

25   by the coverage with fascia. After hemostasis was confirmed, the incised internal collateral ligament was repaired, and the articular capsule, the subcutaneous tissue, and the epidermis were sutured. A cast was fixed to the knee joint in its incurvation position. The operation was ended.

30

        (Evaluation method)

Visual inspection and histological study were performed.

- 158 -

(Results)

Four weeks after operation, the animals receiving the synthetic tissue was significantly repaired according to visual finding (Figure **31**) and histological finding (Figure **32**).

Remarkably, an eosin positive result was observed in the synthetic tissue four weeks after implantation. Also, the formation of a meniscus tissue-like matrix was observed and the biological integration of the synthetic tissue and its adjacent meniscus tissue was completed.

(Example 10: Repair of pig tendon/ligament tissues)

Tendon/ligament tissues were subjected to a repair operation. The state of the wound of a tendon/ligament tissue is confirmed. In this case, a portion of synovial cells are collected. The synovial cells are cultured. The cells are used to produce a synthetic tissue using a protocol as described in Example 1.

Next, by operation, the vicinity of the wound site of the tendon/ligament tissue is cut off to obtain a fresh portion, on which the above-described synthetic tissue is in turn placed. In this case, since the synthetic tissue has adhesion molecules, the synthetic tissue is adhered to the portion without suture. The protocol is described below.

(Anesthesia)

A pig 15 to 17 weeks old (LWD ternary hybrid) was intramuscularly injected via the dorsal portion of its neck with 20 mg/kg Ketaral + 10 mg/kg Seractal. Thereafter, an infusion route was provided in the ear vein, and thereafter,

- 158 -

the respiratory tract was secured using endotracheal intubation. Diprivan was continuously administered at a rate of 0.5 mg/kg/hr to maintain anesthesia. An antibiotic (Cefamezin, 1 g) was administered to prevent post-operational infection.

(Operation)

The animal was positioned and an operation portion was cleaned with a sterilized drape. A knee joint was accessed by medial para-patellar approach. After detecting the internal articular capsule, the middle portion of the capsule was dissected. The lower thighs were bent and laterally rotated, and were further pulled forward, so that the anterior horn portion of the internal meniscus was exposed. In this place, a cylinder-shaped cavity (diameter: 6.5 mm) was created using the mosaic plasty DP (Smith & Nephew). The cavity was filled with the synthetic tissue. In order to protect the synthetic tissue until it was accepted, the meniscus was wrapped with a collagen sheet (Nipro) which was fixed by suture. After hemostasis was confirmed, the incised internal collateral ligament was repaired, and the articular capsule, the subcutaneous tissue, and the epidermis were sutured. A cast was fixed to the knee joint in its incurvation position. The operation was ended.

(Evaluation method)

Histological study was performed based on Frank's method (J. Orthop. Res., 13, 923-9, 1995).

(Results)

According to visual finding and histological finding 6 weeks after operation, the group filled with the synthetic tissue had significantly better healing quality.

(Example 11: Repair of a pig bone)

In this example, repair of bone is experimentally
conducted. Using a protocol as described in Example 1,
synovial cells are collected and cultured to produce a
synthetic tissue.

Next, a sheet of this synthetic tissue is applied
to a bone. The synthetic tissue is applied to an affected
portion mainly by covering it over a cortical bone as well
as a periosteum. As a result, it is demonstrated that the
synthetic tissue comprising synovial cells is effective for
repair of a bone. The protocol is described below.

(Anesthesia)

A pig 15 to 17 weeks old (LWD ternary hybrid) was
intramuscularly injected via the dorsal portion of its neck
with 20 mg/kg Ketaral + 10 mg/kg seractal. Thereafter, an
infusion route was provided in the ear vein, and thereafter,
the respiratory tract was secured using endotracheal
intubation. Diprivan was continuously administered at a
rate of 0.5 mg/kg/hr to maintain anesthesia. An antibiotic
(Cefamezin, 1 g) was administered to prevent
post-operational infection.

(Operation)

The animal was positioned and an operation portion
was cleaned with a sterilized drape. A second metatarsal
bone was accessed from a longitudinal incised portion. The
periosteum of the second metatarsal bone was ablated as much
as possible so that the surface of the second metatarsal
bone was exposed. A window of 1.5 cm (horizontal) × 3 cm
(vertical) was created on the surface of the second metatarsal

bone using a chisel. The window was covered with the
outstretched synthetic tissue. After confirming the
attachment of the synthetic tissue, the the subcutaneous
tissue and the epidermis were sutured. A cast is fixed to
the lower thigh. The operation was ended.

(Evaluation method)
Radiography, micro CT, and histology.

(Results)
Four weeks after operation, evaluation confirmed
that osteogenesis was accelerated in the window portion for
the group filled with the synthetic tissue.

(Example 12: Pig fat-derived tissue)
Next, cells derived from adipose tissue were used
to produce a synthetic tissue.

A) Cells were collected as follows.
1) A specimen was removed from the fat-pad of a knee
joint.
2) The specimen was washed with PBS.
3) The specimen was cut into as many pieces as
possible using scissors.
4) 10 ml of collagenase (0.1%) was added to the
specimen, followed by shaking for one hour in a water bath
at 37°C.
5) An equal amount of DMEM (supplement with 10% FBS)
was added, followed by filtration using a 70 µl filter
(available from Millipore or the like).
6) Cells which passed through the filter and residues
which remained on the filter were placed in a 25-cm^2 flask
(available from Falcon or the like) containing 5 ml of DMEM

supplemented with 10% FBS.

7) Cells attached to the bottom of the flask (including mesenchymal stem cells) were removed and subjected to the production of a synthetic tissue as follows.

B) Production of synthetic tissue

Next, the above-described fat-derived cells were used to produce a synthetic tissue. The concentrations of ascorbic acid 2-phosphate were 0 mM (absent), 0.1 mM, 0.5 mM, 1.0 mM, and 5.0 mM. The synthetic tissue was produced in accordance with the above-described method which was used to produce synovial cells (Example 1). Cells were dessimated at an intial concentration of 5×10^4 cells/cm^2. The result is shown in Figure **33**. The cells were cultured for 14 days. A synthetic tissue was also formed from an adipose tissue-derived cell and had as rich fibronectin and vitronectin as the synovial cell-derived synthetic tissue. Collagen I and III were similarly expressed richly.

C) Implantation experiment

Next, the above-described synthetic tissue is subjected to an implantation experiment in Example 8 (cartilage repair) and in Example 9 (meniscus repair). As a result, it is demonstrated that a repairing capability is possessed by the fat-derived synthetic tissue as with a synovial cell-derived synthetic tissue.

D) Differentiation induction of a fat-derived synthetic tissue into bone/cartilage

The synthetic tissue of this example was induced to differentiate into a cartilage or a bone. The results

are shown in Figure **34**. The left portion of the figure
indicates the results of an osteogenesis experiment. The
upper portion indicates a synthetic tissue, while the lower
portion indicates monolayer culture. The synthetic tissue
had a positive reaction to Alizarin Red in an osteogenesis
induction medium. Thus, osteogenesis was confirmed. The
right portion indicates a chondrogenesis induction
experiment. In this experiment, the synthetic tissue was
differentiated with a stimulus due to chondrogenesis
induction medium+BMP-2 into a cartilage-like tissue which
was positive to Alcian blue. Thus, it was demonstrated that
the fat-derived synthetic tissue also has the ability to
differentiate into a bone and a cartilage as with a synovial
cell-derived synthetic tissue.

    (Example 13: Versatility of shape of synthetic
tissue)

    In this example, a difference in function due to the
shape of a synthetic tissue is measured. The synthetic tissue
may be crumpled up and implanted into an affected portion
instead of using a sheet of the synthetic tissue. Thereby,
it is determined whether or not a tailor-made operation can
be conducted, depending on the shape or the like of a wound
portion.

    In this example, it is investigated whether or not
a synthetic tissue can be implanted when it is in the shape
of a ball, a line, or a tube. The synthetic tissue is confirmed
not to require suture, since it has an adhesion molecule.

    (Example 14: Treatment using a synovial cell)
    In this example, a synovial cell is collected from
a patient having an injured meniscus, and it is determined

whether or not the synovial cell can be used to produce a
synthetic tissue.

(Collection of a human synovial cell).

A human patient, who has a clinical symptom is
diagnosed by an imaging technique as having cartilage injury
or meniscus injury, is subjected to arthroscopy under lumber
anesthesia or general anesthesia. In this case, several
milligrams of synovial membrane is collected. The collected
synovial membrane is transferred to a 50-ml centrifuge tube
(manufactured by Falcon) and washed with phosphate buffered
saline (PBS). Thereafter, the sample is transferred to a
10-cm diameter culture dish (Falcon) and is cut into small
pieces using a sterilized blade. Thereafter, 10 ml of 0.1%
collagenase (Sigma) is added to the cut pieces in the dish.
The dish is shaken in a constant temperature bath at 37°C
for 1 hour 30 minutes. To the solution, 10 ml of medium (DMEM,
Gibco) containing self-serum previously collected or bovine
serum (FBS) is added to inactivate the collagenase, followed
by centrifugation at 1500 rpm for 5 minutes to pellet the
cells. Thereafter, 5 ml of the serum-containing medium is
added again. The culture medium is passed through a 70-μl
filter (Falcon). The collected cells are transferred to a
25 cm² flask (Falcon), followed by culture in a CO_2 incubator
at 37°C.

(Subculture of a synovial cell)

During primary culture, medium is exchanged two times
every week. When cells become confluent, the cells are
subcultured. For initial subculture, the medium is
suctioned and thereafter the cells are washed with PBS.
Trypsin-EDTA (Gibco) is added to the cells which are in turn
allowed to stand for 5 minutes. Thereafter, the

- 164 -

serum-containing medium is added and the resultant mixture
was transferred to a 50-ml centrifuge tube (Falcon), followed
by centrifugation at 1500 rpm for 5 minutes. Thereafter,
15 ml of the serum-containing medium is added to the pellet.
5   The cells are placed in a 150-cm^2 culture dish (Falcon).
Subsequent subculture is performed so that the cell ratio
was 1:3. The same procedure is repeated up to 4 to 5 passages.

(Production of a synthetic tissue)
10   The synovial cell of 4 to 5 passages is treated with
trypsin-EDTA. The synovial cells (4.0×10^6) are dispersed
in 2 ml of medium containing 0.2 mM ascorbic acid 2-phosphate
on a 35-ml culture dish (Falcon), followed by culture in
a CO_2 incubator at 37°C for 7 days. As a result, a culture
15   cell-extracellular matrix complex is formed. The complex
is mechanically detached from the culture dish by pipetting
the periphery thereof two or more hours before an implantation
operation.   After   detachment,   the   culture
cell-extracellular matrix complex contracts into a
20   three-dimensional tissue having a diameter of about 15 mm
and a thickness of about 0.1 mm.

(Example 15: Production of a synthetic tissue from
a human adipocyte)
25   A collection-intended site (e.g., around a knee
joint) from a patient under local anesthesia is resected.
Several milligrams of adipocytes are collected from the site.
The collected adipocytes were treated in a manner similar
to that of the synovial cells. As a result, a
30   three-dimensional synthetic tissue can be produced.

(Example 16: Implantation of a synthetic tissue into
a joint cartilage injury portion)

The synthetic tissue produced in Example 14 or 15 is used for actual implantation. A human subject is subjected to lumbar anesthesia or general anesthesia. Thereafter, the inside of a joint is opened at minimum incision for arthroscopy. After detecting a cartilage injury portion, the size of the cartilage injury is measured. A circular portion of the cartilage is dissected from the bone-cartilage interface using the mosaic plasty harvesting system (Smith and Nephew) and a dental explorer, where the circular portion fully contains the injured cartilage. The synthetic tissue was implanted into the cavity in a portion of cartilage. The synthetic tissue is adhered to the base of the cavity several minutes after implantation. When an affected portion receives a high mechanical stress, the fixation of the synthetic tissue may be reinforced using fibrin glue (initial fixation is reinforced). The present invention is not limited to this. After fixation, the articular capsule, the subcutaneous tissue, and the skin are sutured collectively. After closing the incision site, the joint is fixed using a cast or an orthosis for 2 to 3 weeks. Thereafter, rehabilitation is started within a limited range of motion. When an affected portion is present in a weight-bearing joint (e.g., a knee, a ankle joint, etc.). A full load is able to be applied after 6 to 8 weeks.

As a result, symptoms are cured or ameliorated as follows: a reduction in joint pain when a load or an exercise is applied; elimination of joint effusion; recovery of a joint range of motion; recovery of muscle strength around the joint; prevention of osteoarthritis; and the like. Thus, it is observed that the synthetic tissue of the present invention has no significant side effects and improves the function of a repaired portion.

(Example 17: Implantation into a meniscus injury
portion)

In this example, the synthetic tissue produced in
Example 14 or 15 is actually implanted into a meniscus injury
portion.

A meniscus injury portion is detected in a human
subject under lumbar anesthesia or general anesthesia, using
an arthroscope. A rupture portion of an injury meniscus is
filled with the synthetic tissue. Thereafter, the injured
meniscus and the synthetic tissue are sutured together. All
surgical procedures are performed under an arthroscope.
After surgery, a knee orthosis is used for 2 to 3 weeks.
Thereafter, rehabilitation is started within a limited range
of motion. A full weight bearing is permitted after 5 to
6 weeks.

As a result, symptoms are cured or ameliorated as
follows: a reduction in joint pain when a load or an exercise
is applied to the knee joint; elimination of hydrarthrosis;
recovery of a joint range of motion; recovery of muscle
strength around the joint; recovery of activity; doing sports
again; and the like. Thus, it is observed that the synthetic
tissue of the present invention has no significant side
effects and improves the function of a repaired portion.

(Example 18: Implantation into an Achilles tendon)

The synthetic tissue produced in Example 14 or 15
is implanted into an Achilles tendon injury portion.

A human subject under lumbar anesthesia or general
anesthesia is subjected to Achilles tendon by para-tendon

approach. The portion of degenative tear is detected and
then curetted. The synthetic tissue is implanted into the
portion of degenerative tear. After implantation,
conventional tendon repair is performed. In addition, the
5   surface layer of the repaired portion is covered with the
synthetic tissue, which is in turn sutured and fixed thereto.
After closing the incision site, a cast is fixed to the lower
limb for 4 weeks. A full weight bearing is permitted after
6 to 8 weeks.

10
As a result, symptoms are cured or ameliorated as
follows: recovery of activity level (from walking to a sport
level); a reduction in pain; and a decrease in possibility
of re-rupture. Thus, it is observed that the synthetic tissue
15   of the present invention has no significant side effects
and improves the function of a repaired portion.

(Example 19:   Treatment   of   intractable
pseudarthrosis)
20   In this example, intractable pseudarthrosis is
treated using the synthetic tissue produced in Example 14
or 15. A feature of intractable pseudarthrosis is that a
periosteum, which is a source of supplying cells in a bone
fracture therapy, is severely damaged and lost.
25   Implantation of the synthetic tissue is considered to be
approparate in such a case.

A bone fracture portion is opened in a human subject
under anesthesia. Thereafter, the bone fracture portion is
30   curetted. After the remaining portion is fixed with a plate
or an intramedullary nail, the injured periosteum is covered
with the synthetic tissue. The synthetic tissue is sutured
and fixed to adjacent periosteum tissue. After closing the

- 168 -

incision site, the joint adjacent to the bone fracture portion
is fixed with a cast for 3 to 4 weeks. In the case of a lower
limb bone, full weight bearing is permitted after 6 to 8
weeks.

5

As a result, symptoms are cured or ameliorated as
follows: elimination of pain; recovery of muscle strength
around the joint; and recovery of an activity level. Thus,
it is observed that the synthetic tissue of the present
10 invention has no significant side effects and improves the
function of a repaired portion.

(Example 20: Implantation into a rotator cuff injury
portion)
15 In this example, a synthetic tissue is implanted into
a rotator cuff injury portion. The synthetic tissue is
produced as described in Example 1. Under general
anesthesia, the rotator cuff injury portion is detected by
transdeltoid approach.

20

After detecting the rotator cuff injury portion, the
portion is curetted and is subjected to a typical rotator
cuff repair operation. Thereafter, the surface layer of the
repaired rotator cuff portion is covered with the synthetic
25 tissue. After closing the incision site, the shoulder joint
is fixed with an orthosis for 2 to 3 weeks. Thereafter,
rehabilitation is started within a limited range of motion.
After 6 weeks, full range of motion is permitted.

30 As a result, symptoms are cured or ameliorated as
follows: remission of shoulder pain (particularly, night
pain); recovery of a joint range of motion; recovery of muscle
strength around the shoulder; and recovery of activity. Thus,

it is observed that the synthetic tissue of the present
invention has no significant side effects and improves the
function of a repaired portion.

(Example 21: Study on the possibility of cell
differentiation induction before and after production of
a synthetic tissue)

In this example, a synthetic tissue is produced using
a human synovial cell.

The production process of the synthetic tissue using
a human synovial cell is shown in the upper portions of
Figures 35 and 36. Figure 35 shows production of a synthetic
tissue after a human synovial cell is subjected to
differentiation induction. Figure 36 shows that a
synthetic tissue is produced before the tissue is subjected
to differentiation induction. The differentiation
induction is performed by culturing a human synovial cell
in DMEM medium containing 0.1 μM dexamethasone, 10 mM
β-glycerophosphate, and 50 μg/ml ascorbic acid 2-phosphate
for 14 days. The synthetic tissue is stained with Alzarin
red and alkali phosphatase (ALP). The results of the staining
are shown in the lower portions of Figures 35 and 36. As
can be seen from Figure 35, in either case, the synthetic
tissue is produced and exhibits an osteogenic reaction
positive to the Alzarin red and ALP staining. Therefore,
it is demonstrated that the differentiation induction of
a tissue can be performed either before or after production
of a synthetic tissue.

(Example 22: Study on timing of differentiation for
production of a synthetic tissue in the case of human cells)

In this example, a synthetic tissue was produced using

cells derived from adipose tissue.

A) The cells were collected as follows.

1) A specimen was collected from a fat-pad of a knee
joint.

2) The specimen was washed with PBS.

3) The specimen was cut into as many pieces as
possible.

4) 10 ml of collagenase (0.1%) was added, followed
by shaking in 37°C water bath for one hour.

5) An equal amount of DMEM (supplemented with 10%
FBS) was added. The resultant mixture was passed through
a 70-μl filter (available from Millipore, etc.).

6) Cells passing through the filter and cells
remaining on the filter were cultured in 25-cm² flask
containing 5 ml of DMEM medium supplemented with 10% FBS.

7) The cells (including a mesenchymal stem cell)
attached to the base of the flask were used to produce a
synthetic tissue as follows.

B) Production of a synthetic tissue

Next, the fat-derived cells were used to produce a
synthetic tissue. Ascorbic acid 2-phosphate was used at a
concentration of 0 mM (absence), 0.1 mM, 0.5 mM, 1.0 mM,
or 5.0 mM. The production was conducted in accordance with
the method for producing a synthetic tissue from a synovial
cells (Example 1). The cells were disseminated at an initial
density of 5×10^4 cells/cm².

The cells were used to study the importance of the
differentiation timing using the conditions as described
in Example 21.

As a result, it was similarly demonstrated that the differentiation timing has no particular influence on the adipocyte-derived synthetic tissue of the present invention.

(Example 23:   Confirmation   of   biological integration)

It is known that conventional collagen gel does not always achieve biological integration after implantation. In this example, a conventional collagen gel (3% type I collagen, Koken, Tokyo, Japan) was used. Synovial cells (1×10^5 cells/ml) were embedded in the gel. The resultant gel was implanted into a cavity in a portion of cartilage. As a result, as can be seen from Figure **37**, the integration between the collagen gel and its adjacent cartilage was insufficient, so that a crack was observed (arrow in Fig 37).

On the other hand, when a synthetic tissue of the present invention as produced in Example 1 is introduced into a pig, biological integration is histologically established as shown in Figure **38**.

(Example 24: Study on conditions for detachment during production of a synthetic tissue)

In this example, it was determined whether or not chemical detachment can be used instead of physical detachment (mechanical detachment (e.g., pipetting, etc.)) during the production of the synthetic tissue of the present invention.

(Conditions for culture)
Cell density: 4×10^4 cells/cm^2
Conditinos: CO_2 5%, air 95%, 37°C

Medium: DMEM/F12 (FBS 10%) supplemented with
10 ng/ml TGFβ1.

This medium was used to conduct culture under the
conditions described in Examples 14 and 15 to produce a
synthetic tissue.

When TGF-β was added, the monolayer culture cells
could be more easily detached from the culture dish.

Medium: DMEM (GIBCO), FBS (HyClone) 10%, ITS+Premix
(insulin, transferrin, selenious acid) (BD Biosciences)
6.25 µg/ml, dexamethasone (Sigma) 10^{-7} M, ascorbic acid
(WAKO) 50 µg/ml, pyruvic acid (SIGMA) 100 µg/ml.

The results are shown in Figures **19** and **39**. The
rightmost column in Figure **19** shows the case where TGF-β
was added. In this case, cells were detached from a culture
dish during monolayer culture. Therefore, a synthetic
tissue could not be satisfactorily produced. Figure **39**
shows the result of a tissue which was detached without a
physical stimulus when TGF-β was added in monolayer culture.
These results indicate that TGF-β has the effect of detaching
culture cells.

(Example 25: Actin regulatory agent)
Dihydrocytochalasin B and Y27632 (Yamanouchi
Pharmaceutical), which are known to have an actin
depolymerizing function, were used to study their influence
on the contraction of a synthetic tissue.

A synovium-derived synthetic tissue was produced by
monolayer culture. The tissue was detached from a culture

dish. The tissue was cultured in medium in the presence of
dihydrocytochalasin B (3µM) and Y27632 (10µM). The
transition of the radius of the tissue is shown every unit
culture time in Figure **40**. As can be seen from the figure,
5   contraction was inhibited by the addition of these actin
depolymerizing agents. Dihydrocytochalasin B and Y27632
are   representative   exemplary   actin   polymerization
inhibitors. It will be understood by those skilled in the
art that other actin polymerization inhibitors, such as
10 cytochalasin D and the like, have a similar function.

        (Example 26:   Production   of   an   artificial
bone/cartilage column as a complex of a synthetic tissue
and an artificial bone)

15   A 12-well culture dish was used to produce a synthetic
tissue.

        A column-like artificial bone (NEO BONE: MMT) having
a diameter of 5 mm × 6 mm was placed in a 96-well culture
20 dish. The synthetic tissue was implanted onto the artificial
bone. 100 µl of medium (DMEM, 10% FBS) was placed in each
well of the dish, followed by culture for 2 hours. As a result,
the synthetic tissue was attached to the artificial bone,
thereby obtaining a tissue complex.

25

        This complex was cultured in cartilage induction
medium (DMEM, 10% FBS, ITS+Premix, sodium pyruvate, ascorbic
acid 2-phosphate, 500 ng/ml BMP-2) for 14 days.

30   The result is shown in Figure **41**.

        As can be seen from Figure **41**, it is demonstrated
that the synthetic tissue of the present invention was

satisfactorily adhered to the other synthetic tissue (i.e.,
the artificial bone). Therefore, it will be understood that
the synthetic tissue of the present invention can be combined
with other synthetic tissues into a tissue complex.

5

        (Example 27: Composite tissue obtained by attaching
a synthetic tissue to a collagen scaffold)

        In this example, a microfibrous collagen medical
device (specifically, a collagen synthetic tissue (CMI
10   (Collagen Meniscal Implant) collagen sponge, Amgen, USA))
was attached to a synthetic tissue instead of NEO BONE in
Example 26. The result is shown in Figure **42** (enlarged
photograph). The synthetic tissue of the present invention
is observed to be biologically integrated with the surface
15   of the CMI. Thus, it was demonstrated that a microfibrous
collagen medical device, which is a conventional synthetic
tissue, can be combined with the synthetic tissue of the
present invention to obtain a tissue complex.

20   (Example 28: Production of a synthetic tissue using
a myoblast)

        In this example, an influence of ascorbic acid or
a derivative thereof on the production of a synthetic tissue
when a myoblast was used, was studied. The synthetic tissue
25   was produced as in Example 1.

        After the myoblast was well grown, 5×10^6 myoblast
cells were cultured to form a synthetic tissue. For culture,
SkBM Basal Medium (Clonetics (Cambrex)) was used. Next,
30   ascorbic acid 2-phosphate (0.5 mM), a magnesium salt of
ascorbic acid 1-phosphate (0.1 mM), and L-ascorbic acid Na
(0.1 mM) were added to the medium. After four days of culture,
the tissue was detached. As a control, a synthetic tissue

was produced in medium without ascorbic acids.

(Results)

When ascorbic acids were used, the synthetic tissue
was easily detached as compared to when the ascorbic acid-free
culture system was used. Also, in the ascorbic acid-free
culture system, the tissue was cultured to about several
millimeters. When the tissue exceeded such a level, a crack
or the like occurred in the tissue so that the tissue did
not grow satisfactorily. In addition, it was substantially
difficult to detach the tissue. Thus, no implantable
synthetic tissue was produced (Figure 43). In contrast, the
synthetic tissue of the present invention, which was cultured
in medium containing ascorbic acids, was grown to a size
which allows implantation, and was easily isolated
(Figure 44). Biological integration was investigated, so
that extracellular matrices were highly interacted
(Figure 45).

(Example 29: Effect of a synthetic tissue in the
presence of ascorbic acids)

The synthetic tissue of Example 28, which was
produced in the presence of ascorbic acids, was implanted
into a dilated cardiomyopathy rat. In 28 rats, the left
anterior descending (LAD) was ligated for two weeks to produce
injured hearts. The synthetic tissue of the present
invention was implanted into some of the injured hearts,
while the synthetic tissue of the present invention was not
implanted into the other injured hearts. As controls, rats
without injury to their hearts were obtained.

The rats were anesthetized and operated. The heart
function of the rats was monitored on Day 14 and 28 after

surgery. A ultrasonic instrument (Sonos 5500) having an annular array converter operating at 12 MHz was used to perform endocardiography. Parasternal minor axis imaging and parasternal major axis imaging were performed in a B-imaging mode and an M-imaging mode. In addition to the anterior wall pressure, general parameters (e.g., left ventricular telediastolic diameter, left ventricular telesystolic diameter, internal diameter contraction rate, and ejection fraction) were measured.

Two and four weeks after implantation, the rats were sacrificed with an excessive amount of pentobarbital. The heart was dissected, fixed with 10% formalin, and embedded in paraffin. In a low temperature bath, the heart was cut along the longitudinal axis thereof from the base to the apex to prepare a series of sections having a thickness of 5 mm. Thereafter, the sections were treated for standard histology.

All of the rats with implants were completely cured, and survived for substantially the same period of time as normal rats. Therefore, it was demonstrated that the present invention can completely cure diseases, which are conventionally said to be intractable, in the presence of a specific ECM synthesis promoting agent.

(Example 30: Combined therapy)

A combined therapy of the synthetic tissue produced in the examples and a gene therapy was performed. The combined therapy was intended to promote vascularization in a portion which a synthetic tissue was implanted; promotion of acceptance of an implanted synthetic tissue; and suppression of cell necrosis in a synthetic tissue.

(Methods)

A hemagglutinating virus of Japan (HVJ)-liposome complex was prepared in accordance with Kaneda Y., Iwai K., Uchida T., Increased expression of DNA co-introduced with nuclear protein in adult rat liver. Science, 1989;243:375-378. The procedure will be briefly described below. A DNA solution (200 μl) was added, followed by shaking for 30 seconds. The solution was allowed to stand at 37°C in a constant temperature bath for 30 seconds. This step was performed 8 times. Thereafter, ultrasonication was performed for 5 seconds, followed by shaking for 30 seconds. BSS (0.3 ml) was added, followed by shaking at 37°C in a constant temperature bath. Inactivated HVJ was added. The mixture was placed on ice for 10 minutes. The mixture was then shaken at 37°C in a constant temperature bath for one hour. A 60% sucrose solution (1 ml) and a 30% sucrose solution (6 ml) were layered in a centrifuge tube. A HVJ liposome solution was placed on top of the layered sucrose solution. Additional BSS was added to the tube. Centrifugation was performed at 62,800 g at 4°C for 1.5 hours. A solution immediately above the 30% sucrose solution layer was recovered. The solution was preserved at 4°C and was used for gene introduction.

About 0.2 ml of Sendai virus liposome-plasmid complex (including 15 μg of human HGF cDNA) was injected into a cardiac infarction region. For a control group, an empty vector was introduced into a heart muscle having infarction. The human HGF concentration of heart tissue was measured with an enzyme linked immunosolvent assay (ELISA) using an anti-human HGF monoclonal antibody (Institite of Immunology, Tokyo, Japan) (Ueda H., Sawa Y., Matsumoto K. et al., Gene Transfection of Hepatocyte Growth Factor

Attenuates reperfusion Injury in the Heart, Ann. Thorac.
Surg., 1999, 67:1726-1731). The synthetic tissue produced
in Example 30 was used. The cardiac infarction models
produced by ligating LAD were subjected to three different
therapies: 1) a cell sheet group; 2) a gene therapy group;
3) a combined therapy group; and 4) a control group. Changes
in heart function and cardiomuscular tissue were studied.

(Results)

For the synthetic tissue implanted group and the
combined therapy group, the contractibility and
expansibility of the heart were ameliorated. In addition,
for the combined therapy group, it can be confirmed that
vasculization was observed in the cardiac infarction portion,
and the acceptance of implanted cells was improved.

(Conclusion)

By combining a synthetic tissue and a gene therapy,
the decreased heart function ameliorating effect, the
vasculization effect, and the cell protecting effect are
obtained, so that a higher level of amelioration of the
decreased heart function can be observed.

Although certain preferable embodiments have been
described herein, it is not intended that such embodiments
be construed as limitations on the scope of the invention
except as set forth in the appended claims. Various other
modifications and equivalents will be apparent to and can
be readily made by those skilled in the art, after reading
the description herein, without departing from the scope
and spirit of this invention. All patents, published patent
applications and publications cited herein are incorporated
by reference as if set forth fully herein.

INDUSTRIAL APPLICABILITY

The present invention usefully provides a basic therapeutic method, technique, pharmaceutical agent, and medical device for diseases which are conventionally difficult to treat. Particularly, the present invention provides an epoch-making therapy and prevention because it promotes recovery to a substantially native state. The present invention also provides a pharmaceutical agent, cell, tissue, composition, system, kit, and the like, which are used for such an epoch-making therapy and prevention.

There is a demand for repair and regeneration of joint tissues, mainly including bones and cartilages which are targeted by the present invention. The number of bone fracture patients, which are targeted by bone regeneration, accounts for several hundreds of thousands per year. It is also said that there are 30 million potential patients having osteoarthritis which is targeted by the cartilage regenerative therapy. Thus, the potential market is huge. The present invention is also highly useful for peripheral industries. Acute competition has been started in the regenerative medical research on joint tissues, mainly including bone and cartilage. The synthetic tissue of the present invention is a safe and original material made of cells collected from an organism, such as a patient or the like, and is highly useful in view of the lack of side effects or the like.

- 180 -

CLAIMS

1. An implantable synthetic tissue.

2. A synthetic tissue according to claim 1, which is biologically organised in the third dimensional direction.

3. A synthetic tissue according to claim 1, which has biological integration capability with surroundings.

4. A synthetic tissue according to claim 3, wherein the biological integration capability includes capability to adhere to surrounding cells and/or extracellular matrices.

5. A synthetic tissue according to claim 1, which comprises cells.

6. A synthetic tissue according to claim 1, which is substantially made of cells and a material derived from the cells.

7. A synthetic tissue according to claim 1, which is substantially made of cells and an extracellular matrix (ECM) derived from the cells.

8. A synthetic tissue according to claim 7, wherein the extracellular matrix contains at least one selected from the group consisting of collagen I, collagen III, vitronectin and fibronectin.

9. A synthetic tissue according to claim 7, wherein the extracellular matrix contains collagen I, collagen III, vitronectin and fibronectin.

10. A synthetic tissue according to claim 7, wherein the extracellular matrix contains vitronectin.

11. A synthetic tissue according to claim 7, wherein the extracellular matrix contains fibronectin.

12. A synthetic tissue according to claim 7, wherein the extracellular matrix contains collagen I and collagen III, the collagen constitutes 5% to 25% of the tissue, and the ratio of the collagen I to the collagen III is between 1:10 and 10:1.

13. A synthetic tissue according to claim 7, wherein the extracellular matrix and the cells are integrated together into a three-dimensional structure.

14. A synthetic tissue according to claim 7, wherein the extracellular matrix is diffusedly distributed in the tissue.

15. A synthetic tissue according to claim 1, wherein an extracellular matrix is diffusedly distributed, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm^2 in the tissue have a ratio within a range of about 1:3 to about 3:1.

16. A synthetic tissue according to claim 1, which is heterologous, allogenic, isologous, or autogenous.

17. A synthetic tissue according to claim 1, which is free of scaffolds.

18. A synthetic tissue according to claim 1, which is used

to implant cells.

19. A synthetic tissue according to claim 1, which is large
sized.

20. A synthetic tissue according to claim 1, which has a
volume of at least about 20 mm^3.

21. A synthetic tissue according to claim 1, which is
flexible.

22. A synthetic tissue according to claim 1, which is
expandable and contractile.

23. A synthetic tissue according to claim 1, which can
withstand heart pulsation.

24. A synthetic tissue according to claim 1, which is
biologically organized in all three dimensional directions.

25. A synthetic tissue according to claim 24, wherein the
biological integration is selected from the group consisting
of internal binding of extracellular matrix, electrical
integration, and intercellular signal transduction.

26. A synthetic tissue according to claim 1, which has a
tissue strength which allows the synthetic tissue to be
clinically applicable.

27. A synthetic tissue according to claim 26, wherein the
strength is a break strength of about 0.02 N to about 2 N.

28. A synthetic tissue according to claim 26, wherein the

tissue strength is sufficient to provide self-supporting
ability.

5   29. A synthetic tissue according to claim 28, wherein the
self-supporting ability is characterized in that the
synthetic tissue is not substantially broken when the
synthetic tissue is picked up using forceps having a tip
area of 0.05 to 3.0 mm^2.

10   30. A synthetic tissue according to claim 28, wherein the
self-supporting ability is characterized in that the
synthetic tissue is not broken when the synthetic tissue
is picked up with a hand.

15   31. A synthetic tissue according to claim 26, wherein the
site to which the synthetic tissue is intended to be applied,
includes a heart.

32. A synthetic tissue according to claim 26, wherein the
20   site to which the synthetic tissue is intended to be applied,
includes an intervertebral disk, a meniscus, a cartilage,
a bone, a ligament, or a tendon.

33. A synthetic tissue according to claim 26, wherein:
25   the synthetic tissue is a cartilage, an
intervertebral disk, a meniscus, a ligament, or a tendon;
and
        the synthetic tissue remains attached without an
additional fixation procedure, after the synthetic tissue
30   is implanted into an injured portion of the intra-articular
tissue.

34. A method for producing a synthetic tissue, comprising

the steps of:

A) providing cells;

B) placing the cells in a container, the container having cell culture medium containing an ECM synthesis promoting agent and having a sufficient base area which can accommodate a synthetic tissue having a desired size;

C) culturing the cells in the container along with the cell culture medium containing the ECM synthesis promoting agent for a period of time sufficient for formation of the synthetic tissue having the desired size; and

D) detaching the cells from the container.

35. A method according to claim 34, wherein a stimulus for inducing tissue contraction is applied in the detaching step.

36. A method according to claim 35, wherein the stimulus includes a physical or chemical stimulus.

37. A method according to claim 36, wherein the physical stimulus includes shaking of the container, pipetting, or deformation of the container.

38. A method according to claim 34, wherein the detaching step includes adding an actin regulatory agent.

39. A method according to claim 38, wherein the actin regulatory agent includes a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.

40. A method according to claim 39, wherein the actin depolymerizing agent is selected from the group consisting of Slingshot, cofilin, cyclase associated protein (CAP),

actin interacting protein 1 (AIP1), actin depolymerizing factor (ADF), destrin, depactin, actophorin, cytochalasin, and NGF (nerve growth factor).

41. A method according to claim 39, wherein the actin polymerizing agent is selected from the group consisting of RhoA, mDi, profilin, Rac1, IRSp53, WAVE2, ROCK, LIM kinase, cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, lysophosphatidic acid (LPA), insulin, platelet derived growth factor (PDGF) a, PDGFb, chemokine, and transforming growth factor (TGF) β.

42. A method according to claim 34, wherein the container is free of scaffolds.

43. A method according to claim 34, wherein the cells are first cultured in monolayer culture.

44. A method according to claim 34, wherein the ECM synthesis promoting agent includes TGFβ1, TFGβ3, ascorbic acid, ascorbic acid 2-phosphate, or a derivative or salt thereof.

45. A method according to claim 44, wherein the ascorbic acid, ascorbic acid 2-phosphate, or the derivative or salt thereof is present at a concentration of at least 0.1 mM.

46. A method according to claim 44, wherein the TGFβ1 or TFGβ3 is present at a concentration of at least 1 ng/ml.

47. A method according to claim 34, wherein the cells are placed at a concentration of 5×10^4 to 5×10^5 cells per 1 cm^2, and the ECM synthesis promoting agent is ascorbic acid, ascorbic acid 2-phosphate, or a derivative or salt thereof.

and the ascorbic acid, ascorbic acid 2-phosphate, or the
derivative or salt thereof is provided at a concentration
of at least 0.1 mM.

5   48. A method according to claim 34, further comprising
causing the synthetic tissue to detach from the container
and self-contract.

49. A method according to claim 48, wherein the detaching
10   and self-contraction are achieved by providing a physical
stimulus to the container.

50. A method according to claim 48, wherein the detachment
and self-contraction are achieved by providing a chemical
15   stimulus to the container.

51. A method according to claim 34, wherein the sufficient
period of time is at least 3 days.

20   52. A method according to claim 34, wherein the sufficient
period of time is at least 3 days and a period of time required
for the synthetic tissue to be spontaneously detached from
the container at a maximum.

25   53. A method according to claim 52, wherein the period of
time required for the synthetic tissue to be spontaneously
detached from the container is at least 40 days.

54. A method according to claim 34, further comprising:
30   causing the synthetic tissue to differentiate.

55. A method according to claim 54, wherein the
differentiation includes osteogenesis, chondrogenesis,

adipogenesis, tendon differentiation, and ligament
differentiation.

56. A method according to claim 55, wherein the osteogenesis
is performed in medium containing dexamethasone,
β-glycerophosphate, and ascorbic acid 2-phosphate.

57. A method according to claim 56, wherein the medium
contains at least one selected from the group consisting
of BMP (bone morphogenetic protein)-2, BMP-4, and BMP-7.

58. A method according to claim 55, wherein the
chondrogenesis is performed in medium containing pyruvic
acid, dexamethasone, ascorbic acid 2-phosphate, insulin,
transferrin, and selenious acid.

59. A method according to claim 58, wherein the medium
contains at least one selected from the group consisting
of BMP-2, BMP-4, BMP-7, TGF (transforming frowth factor)-β1
and TGF-β3.

60. A method according to claim 54, wherein the
differentiation step is performed before or after the
detaching step.

61. A method according to claim 54, wherein the
differentiation step is performed after the detaching step.

62. A method according to claim 34, wherein the cell includes
cells of 3 or more passages.

63. A method according to claim 34, wherein the cells include
cells of 3 to 8 passages.

64. A method according to claim 34, wherein the cells are
provided at a cell density of 5.0×10^4 to 5.0×10^6 cells/cm^2.

65. A method according to claim 34, wherein the cells include
myoblasts.

66. A method according to claim 34, wherein the cells include
fat-derived cells.

67. A method according to claim 34, wherein the cells include
synovium-derived cells.

68. A method according to claim 34, wherein the cells include
mesenchymal stem cells.

69. A method according to claim 68, wherein the mesenchymal
stem cells are derived from an adipose tissue, a synovial
membrane, a tendon, a bone, or a bone marrow.

70. A method according to claim 34, further comprising:
        producing a plurality of the synthetic tissues and
attaching the plurality of the synthetic tissues together
to be integrated.

71. A cell culture composition for producing a synthetic
tissue from cells, comprising:
        A) an element for maintaining the cells; and
        B) an extracellular matrix synthesis promoting
agent.

72. A method according to claim 68, wherein the ECM synthesis
promoting agent includes TGFβ1, TFGβ3, ascorbic acid,

ascorbic acid 2-phosphate, or a derivative or salt thereof.

73. A method according to claim 72, wherein TGFβ1 or TFGβ3
is present at a concentration of at least 1 ng/ml, or ascorbic
acid, ascorbic acid 2-phosphate, or the derivative or salt
thereof is present at a concentration of at least 0.1 mM.

74. A complex for reinforcing a portion of an organism,
comprising cells and a component derived from the cells.

75. A complex according to claim 74, which has biological
integration capability with surroundings.

76. A complex according to claim 75, wherein the biological
integration capability include capability to adhere to
surrounding cells and/or extracellular matrices.

77. A complex according to claim 74, which is substantially
made of cells and a material derived from the cells.

78. A complex according to claim 74, which is substantially
made of cells and an extracellular matrix derived from the
cells.

79. A synthetic tissue according to claim 78, wherein the
extracellular matrix is selected from the group consisting
of collagen I, collagen III, vitronectin and fibronectin.

80. A complex according to claim 78, wherein the
extracellular matrix and the cells are integrated together
into a three-dimensional structure.

81. A complex according to claim 78, wherein the

extracellular matrix is provided on a surface of the complex.

82. A complex according to claim 78, wherein the
extracellular matrix is diffusedly distributed on a surface
of the complex.

83. A complex according to claim 74, wherein an extracellular
matrix is diffusedly distributed on a surface of the complex,
and the distribution densities of the extracellular matrix
in two arbitrary sections of 1 cm^2 in the complex have a ratio
within a range of about 1:3 to about 3:1.

84. A complex according to claim 78, wherein the
extracellular matrix includes fibronectin or vitronectin.

85. A complex according to claim 74, which is heterologous,
allogenic, isologous, or autogenous.

86. A complex according to claim 74, wherein the portion
includes a bag-shaped organ.

87. A complex according to claim 86, wherein the bag-shaped
organ includes a heart.

88. A complex according to claim 74, wherein the portion
includes a bone or cartilage tissue.

89. A complex according to claim 74, wherein the portion
includes avascular tissue.

90. A complex according to claim 74, wherein the portion
includes an intervertebral disk, a meniscus, a ligament,
or a tendon.

91. A complex according to claim 74, wherein the reinforcement is achieved by replacing the portion with the complex or providing the complex to cover the portion, or both.

92. A complex according to claim 74, which resists the expansion and contraction of the portion.

93. A complex according to claim 74, which has biological integration.

94. A complex according to claim 74, wherein the biological integration selected from the group consisting of internal binding of extracellular matrix, electrical integration, and intercellular signal transduction.

95. A complex according to claim 74, which is formed by culturing cells in the presence of an ECM synthesis promoting agent.

96. A complex according to claim 74, which has self-supporting ability.

97. A method for reinforcing a portion of an organism, comprising the steps of:

A) replacing the portion with a complex comprising cells and a component derived from the cells or providing the complex to cover the portion, or both; and

B) holding the complex for a sufficient period of time for biologically adhering the complex to the portion.

98. A method according to claim 97, wherein the adhesion

is achieved by adhesion between extracellular matrix and extracellular matrix.

99. A method according to claim 97, which has biological integration capability with surroundings.

100. A method according to claim 99, wherein the biological integration capability include capability to adhere to surrounding cells and/or extracellular matrices.

101. A method according to claim 97, which is substantially made of cells and a material derived from the cells.

102. A method according to claim 97, which is substantially made of cells and an extracellular matrix derived from the cells.

103. A method according to claim 102, wherein the extracellular matrix contains one selected from the group consisting of collagen I, collagen III, vitronectin and fibronectin.

104. A method according to claim 102, wherein the extracellular matrix contains all of collagen I, collagen III, vitronectin and fibronectin.

105. A method according to claim 102, wherein the extracellular matrix contains vitronectin.

106. A method according to claim 102, wherein the extracellular matrix contains fibronectin.

107. A method according to claim 97, wherein an extracellular

matrix is provided on a surface of the complex.

108. A method according to claim 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex.

109. A method according to claim 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm^2 have a ratio within a range of about 1:3 to about 3:1.

110. A complex according to claim 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm^2 have a ratio within a range of about 1:2 to about 2:1.

111. A method according to claim 97, which is heterologous, allogenic, isologous, or autogenous.

112. A method according to claim 97, wherein the portion includes a bag-shaped organ.

113. A method according to claim 112, wherein the bag-shaped organ includes a heart.

114. A method according to claim 97, wherein the complex resists the expansion and contraction of the portion.

115. A method according to claim 97, wherein the complex has biological integration.

116. A method according to claim 115, wherein the biological

- 194 -

integration selected from the group consisting of internal
binding of extracellular matrix, electrical integration,
and intercellular signal transduction.

5   117. A method according to claim 97, further comprising:
            forming the complex by culturing the cells in the
     presence of an ECM synthesis promoting agent.

     118. A method according to claim 97, wherein the portion
10   is a heart and the heart has a disease or disorder selected
     from the group consisting of heart failure, ischemic heart
     disease, myocardial infarct, cardiomyopathy, myocarditis,
     hypertrophic cardiomyopathy, dilated phase hypertrophic
     cardiomyopathy, and dilated cardiomyopathy.

15

     119. A method according to claim 97, wherein the portion
     includes an avascular lesion.

     120. A method according to claim 97, wherein the portion
20   includes a vascular lesion.

     121. A method according to claim 97, wherein the portion
     includes a bone or a cartilage.

25   122. A method according to claim 97, wherein the portion
     includes an intervertebral disk, a meniscus, a ligament,
     or a tendon.

     123. A method according to claim 97, wherein the portion
30   includes a bone or a cartilage, and the bone or the cartilage
     is damaged or degenerated.

     124. A method according to claim 97, wherein the portion

includes intractable fracture, osteonecrosis, cartilage
injury, meniscus injury, ligament injury, tendon injury,
cartilage degeneration, meniscus degeneration,
intervertebral disk denaturation, ligament degeneration,
5   or tendon degeneration.

125. A method according to claim 97, wherein the sufficient
period of time is at least 10 days.

10   126. A method according to claim 97, wherein the complex
has self-supporting ability.

127. A method according to claim 97, which has biological
integration capability with surroundings.

15
128. A method according to claim 97, which is substantially
made of cells and an extracellular matrix derived from the
cells.

20   129. A method according to claim 97, further comprising
implanting another synthetic tissue.

130. A method according to claim 129, wherein the other
synthetic tissue is an artificial bone or a microfibrous
25   collagen medical device.

131. A method according to claim 97, which is substantially
made of cells and an extracellular matrix derived from the
cells, wherein the other synthetic tissue is an artificial
30   bone or a microfibrous collagen medical device.

132. A method according to claim 130, the artificial bone
includes hydroxyapatite.

133. A method for treating a portion of an organism, comprising the steps of:

    A) replacing the portion with a complex comprising cells and a component derived from the cells or providing the complex to cover the portion, or both; and

    B) holding the complex for a sufficient period of time for restoring a condition of the portion.

134. A method according to claim 133, wherein the treatment is for the treatment, prevention, or reinforcement of a disease, disorder, or condition of a heart, a bone, a cartilage, a ligament, a tendon, a meniscus, or an intervertebral disk.

135. A method according to claim 133, wherein the complex has self-supporting ability.

136. A method according to claim 133, wherein the complex has biological integration capability with surroundings.

137. A method according to claim 133, wherein the complex is substantially made of cells and an extracellular matrix derived from the cells.

138. A method according to claim 133, further comprising implanting another synthetic tissue in addition to the replacement or coverage of the portion.

139. A method according to claim 138, wherein the other synthetic tissue includes an artificial bone or a microfibrous collagen medical device.

140. A method according to claim 133, which is substantially
made of cells and an extracellular matrix derived from the
cells, wherein the other synthetic tissue includes an
artificial bone or a microfibrous collagen medical device.

141. A method according to claim 139, the artificial bone
includes hydroxyapatite.

142. A method for producing a synthetic tissue, comprising
the steps of:

      A) providing cells;

      B) placing the cells in a container, the container
having cell culture medium containing an ECM synthesis
promoting agent and having a sufficient base area which can
accommodate a synthetic tissue having a desired size;

      C) culturing the cells in the container along with
the cell culture medium containing the ECM synthesis
promoting agent for a period of time sufficient for formation
of the synthetic tissue having the desired size; and

      D) regulating a thickness of the synthetic tissue
by a physical or chemical stimulus to a desired thickness.

143. A method according to claim 142, wherein the physical
stimulus includes shear stress between the synthetic tissue
and the container, deformation of the base of the container,
shaking of the container, or pipetting.

144. A method according to claim 142, wherein the chemical
stimulus is obtained by using a chemical substance selected
from the group consisting of actin depolymerizing agents
and actin polymerizing agents.

145. A method according to claim 144, wherein the actin

depolymerizing agent is selected from the group consisting
of Slingshot, cofilin, CAP (cyclase associated protein),
AIP1 (actin interacting protein 1), ADF (actin depolymerizing
factor), destrin, depactin, actophorin, cytochalasin, and
5   NGF (nerve growth factor).

146. A method according to claim 144, wherein the actin
polymerizing agent is selected from the group consisting
of RhoA, mDi, profilin, Rac1, IRSp53, WAVE2, ROCK, LIM kinase,
10   cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, LPA
(lysophosphatidic acid), insulin, PDGF (platelet derived
growth factor), PDGFb, chemokine, and TGF (transforming
growth factor) β.

15   147. A method according to claim 144, wherein the desired
thickness is regulated by adjusting a ratio of the actin
depolymerizing agent to the actin polymerizing agent.

148. A method according to claim 142, further comprising:
20   producing a plurality of the synthetic tissues and
attaching the plurality of the synthetic tissues together
to be integrated.

149. A tissue complex, comprising an implantable synthetic
25   tissue and another synthetic tissue.

150. A tissue complex according to claim 149, wherein the
implantable synthetic tissue is substantially made of cells
and a material derived from the cells.
30
151. A tissue complex according to claim 149, wherein the
implantable synthetic tissue is substantially made of cells
and an extracellular matrix derived from the cells.

152. A tissue complex according to claim 151, wherein the extracellular matrix is selected from the group consisting of collagen I, collagen III, vitronectin, and fibronectin.

153. A tissue complex according to claim 151, wherein the extracellular matrix contains all of collagen I, collagen III, vitronectin, and fibronectin.

154. A tissue complex according to claim 149, wherein the other synthetic tissue includes an artificial bone or a microfibrous collagen medical device.

155. A tissue complex according to claim 154, the artificial bone includes hydroxyapatite.

156. A tissue complex according to claim 149, the implantable synthetic tissue is biologically integrated with the other synthetic tissue.

157. A tissue complex according to claim 156, wherein the biological integration is achieved via an extracellular matrix.

158. A composition for use in producing a synthetic tissue having a desired thickness, comprising a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.

159. A composition according to claim 158, wherein the actin depolymerizing agent is selected from the group consisting of Slingshot, cofilin, CAP (cyclase associated protein), AIP1 (actin interacting protein 1), ADF (actin depolymerizing

factor), destrin, depactin, actophorin, cytochalasin, and NGF (nerve growth factor).

160. A composition according to claim 158, wherein the actin polymerizing agent is selected from the group consisting of RhoA, mDi, profilin, Rac1, IRSp53, WAVE2, ROCK, LIM kinase, cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, LPA (lysophosphatidic acid), insulin, PDGF (platelet derived growth factor) a, PDGFb, chemokine, and TGF (transforming growth factor) β.

FIG.1

Monolayer — Contraction → Synthetic tissue

100 μm

FIG.2

0 mM   0.1 mM

1 mM   5mM

x200

(5.0x10^5/cm^2)

FIG.3

Day 3

Day 7

Day 14

Day 21

Day 1   It is difficult to detach cell sheet

$(1 \times 10^6 cell/ cm^2 Asc-2P \ 1mM)$

FIG.4

FIG.5
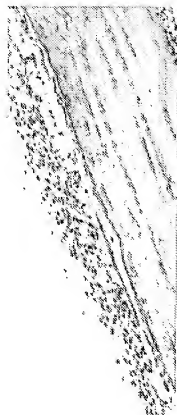
Normal tendon tissue
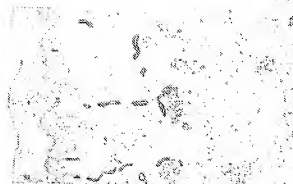
Normal meniscus tissue

Normal cartilage tissue

Normal synovial membrane tissue

Normal skin

FIG.6

HE staining

Negative control

Vitronectin

fibronectin

FIG.7

1.6x10⁶/12well
Culture time: 3,7,14,21day
Asc-2P : 0, 0.1, 1, 5mM

⟶ Determination of Hydroxyproline

□ 0mM
▨ 0.1mM
□ 1mM
□ 5mM

* : $p < 0.05$
** : $p < 0.01$
*** : $p < 0.001$

N=5
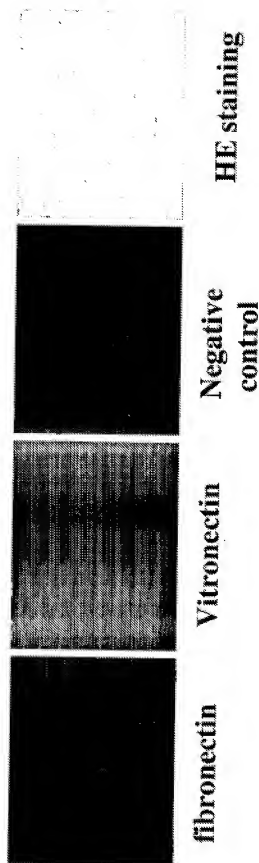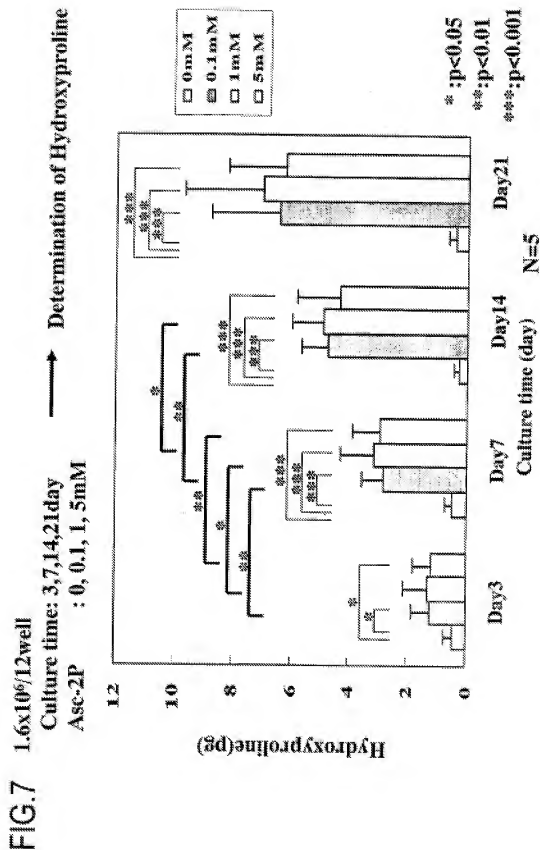
FIG.8

1.6x10⁶/12well   N=5
Culture time: 3,7,14,21day
Asc-2P   : 0, 0.1, 1, 5mM

FIG.9

P=5

2.0×10^6	1.0×10^6
5.0×10^5	2.5×10^5
1.0×10^5	5.0×10^4

P=6

2.0×10^6	1.0×10^6
5.0×10^5	2.5×10^5
1.0×10^5	5.0×10^4

P=7

1.0×10^6	7.5×10^5
5.0×10^5	2.5×10^5
1.0×10^5	5.0×10^4

FIG.10

*:35mm dish
**:60mm dish
***:100mm dish

2cm
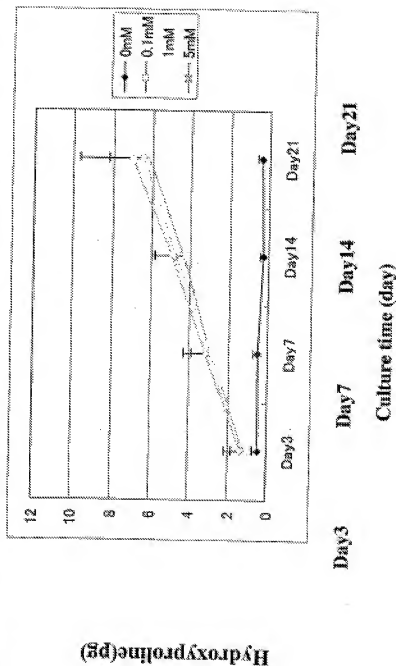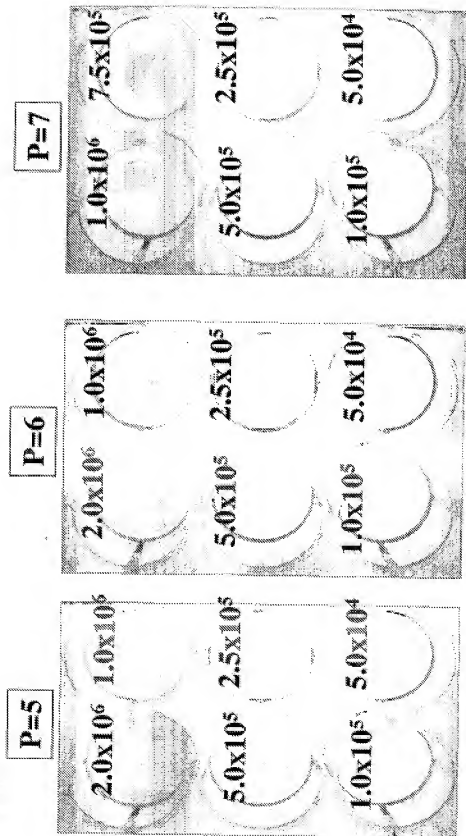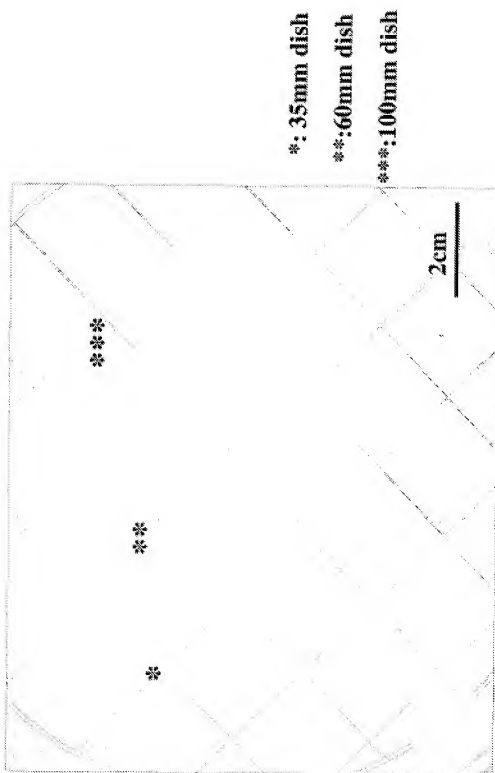
FIG.11
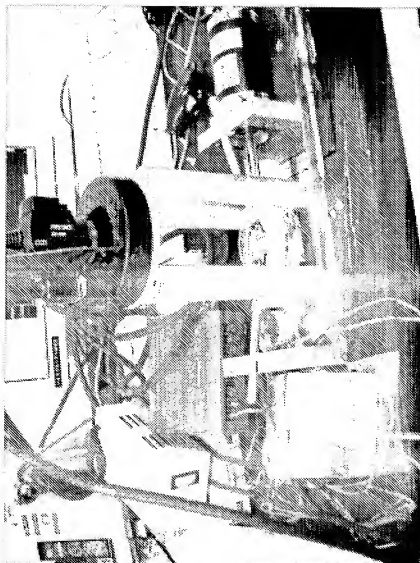
FIG.12
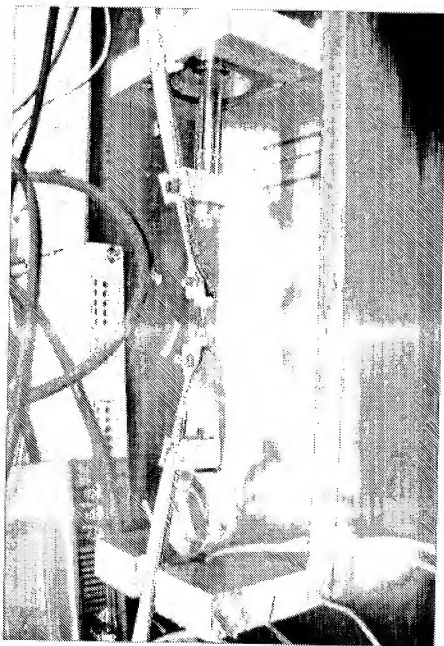
FIG.13
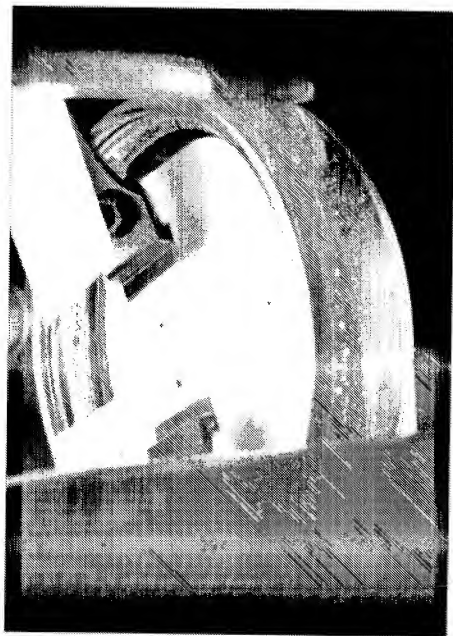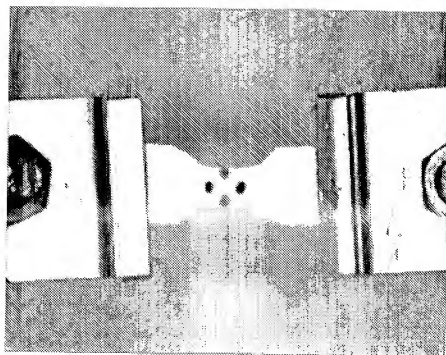
FIG.14

FIG.15

FIG.16

FIG.17

FIG.18

14 day culture

ALP staining

alizarin red staing

control

osteogenesis

control

osteogenesis
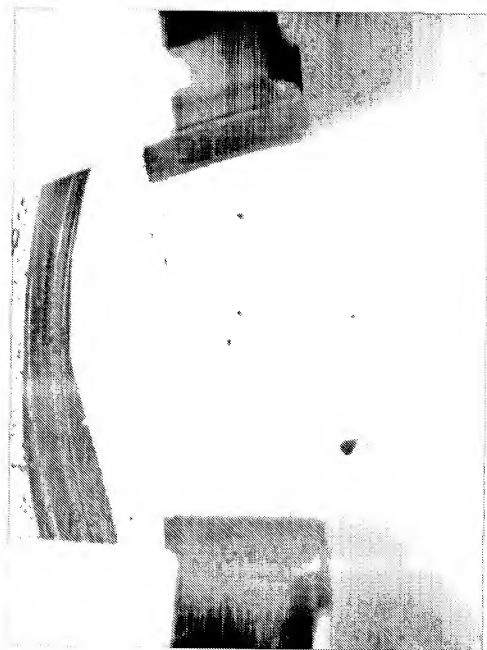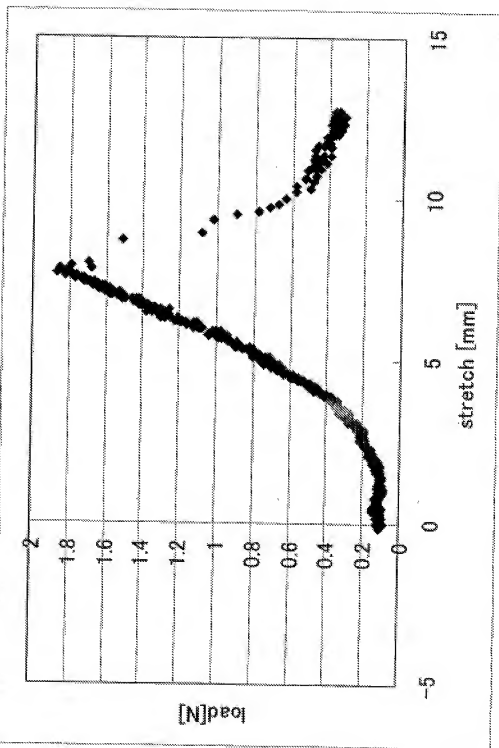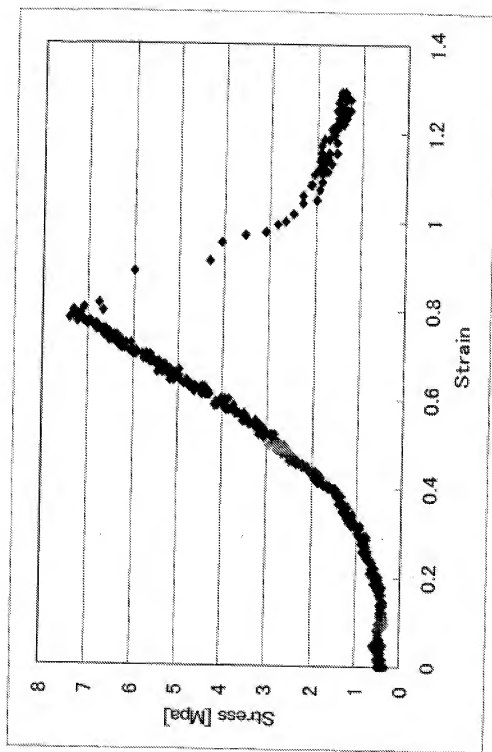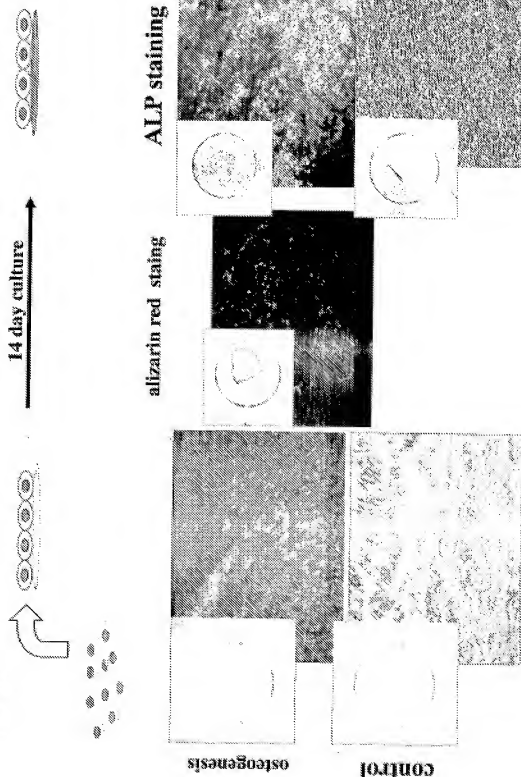
FIG.19
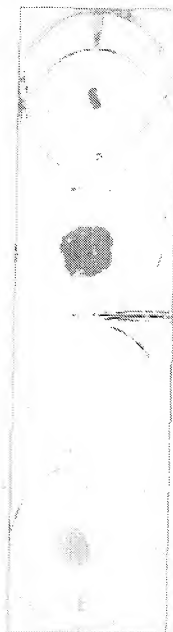
A) Synthetic tissue

B) monolayer

Culture medium
Chondrogenic medium
BMP-2
TGF-b

FIG.20

FIG.21

Aggrecan

Col II

Sox 9

B-actin

Growth medium
Chondrogenic medium
BMP
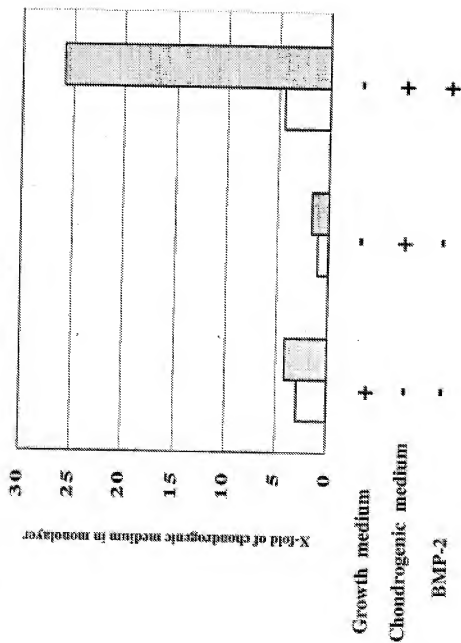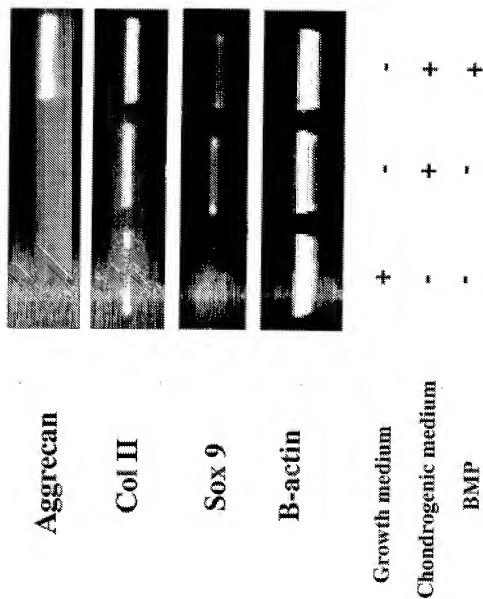
FIG.22

	Aggrecan	Col II	B-actin					
Growth medium				-	+	+	+	-
Chondrogenic medium				-	+	+	-	+
BMP				-	+	-	+	-
				-	+	-	-	+
Synthetic tissue				+	-	-	+	-
monolayer				+	-	-	-	+

FIG.23

remove superficial zone
digested with
chondroitinase ABC

(Hunziker EB. IBJS 1996)

Cultured for
7 days

HE staining

Fibronectin

a

b

x40

x200

FIG.24
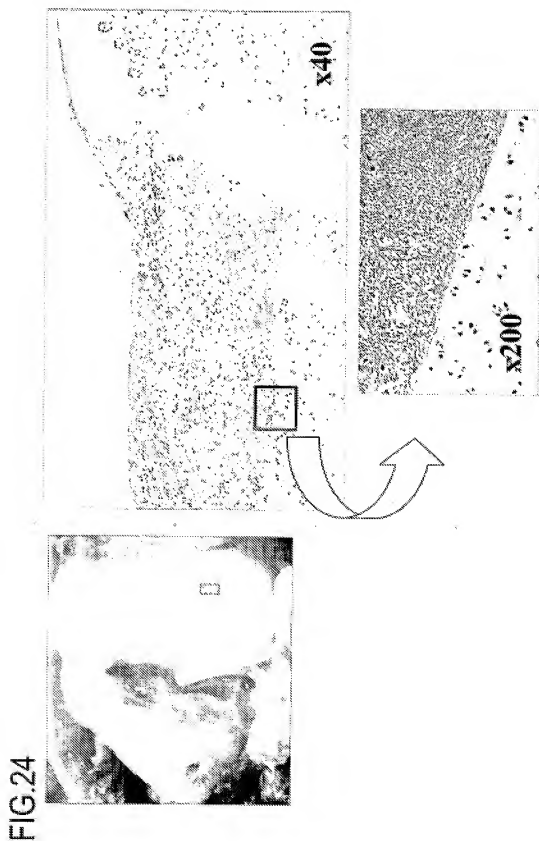
FIG.25
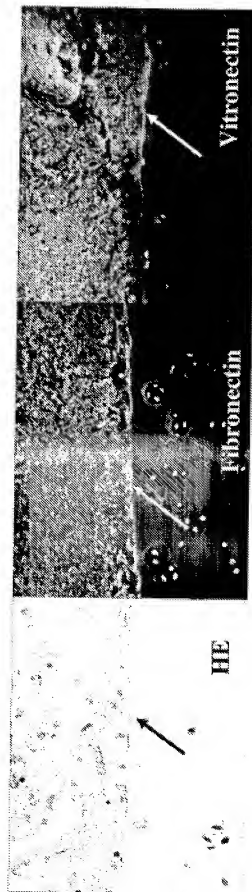
FIG.26
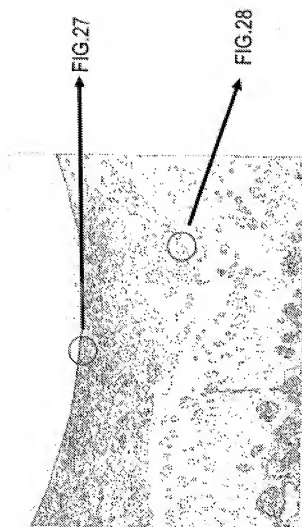
FIG.27

FIG.28

FIG.27

FIG.28
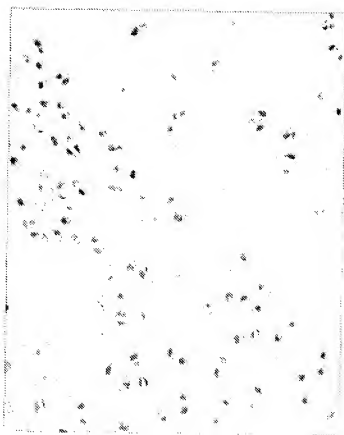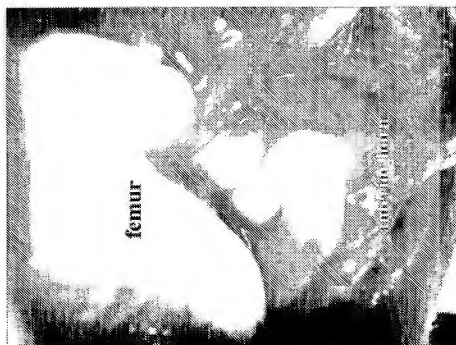
FIG.29

FIG.30

membrana synovialis
derived artificial tissue
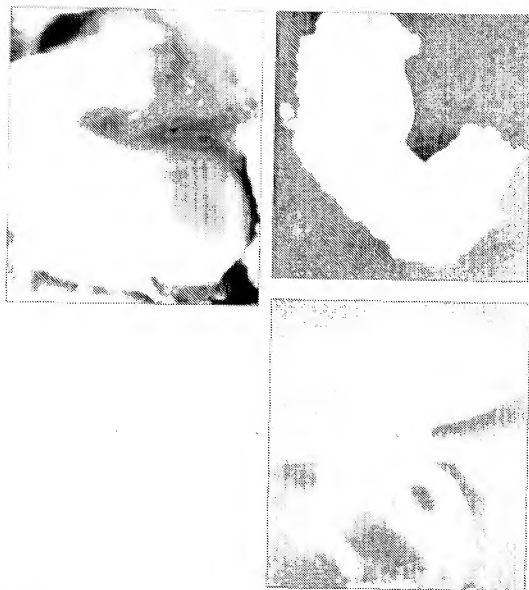
FIG.31

FIG.32
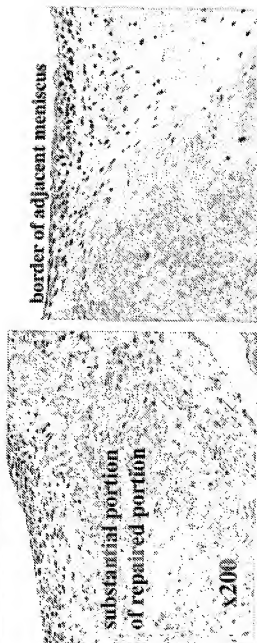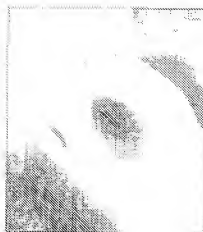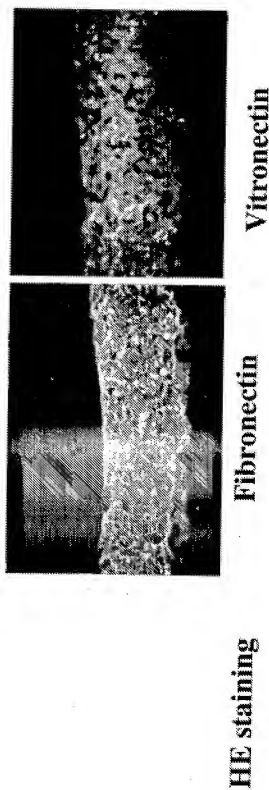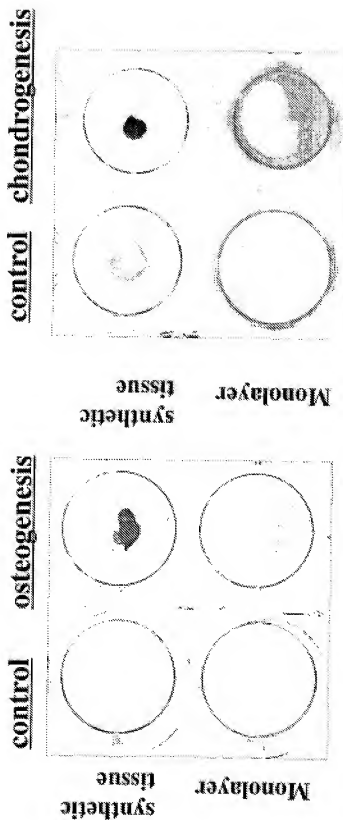
border of adjacent meniscus

substantial portion
of repaired portion

x200

FIG.33

Vitronectin

Fibronectin

HE staining

FIG.34

35/46

FIG.35

FIG.36

FIG.37

FIG.38

Pre-ope

Post-ope

10days

L   M

x40

FIG.39

FIG.40

FIG.41

NEOBONE

NEOBONE +
Synthetic tissue SI

FIG.42

FIG.43

FIG.44

FIG.45

FIG.46

FIG.47

indenter (pad)

test piece

load curve

load removal curve

$$\left[\frac{dF}{dh}\right]_{Fmax}$$

F_{max}

h_r h_{max}

load load F
displacement h

contact depth

test piece
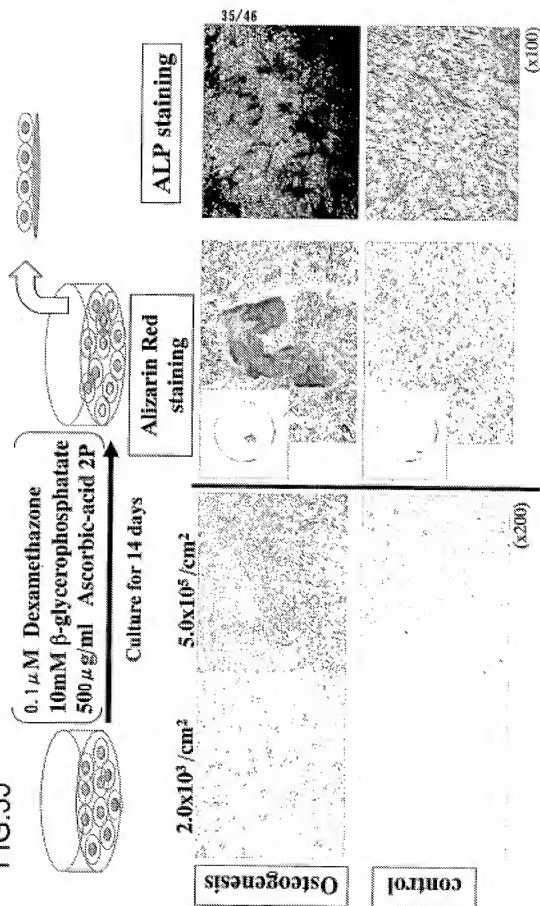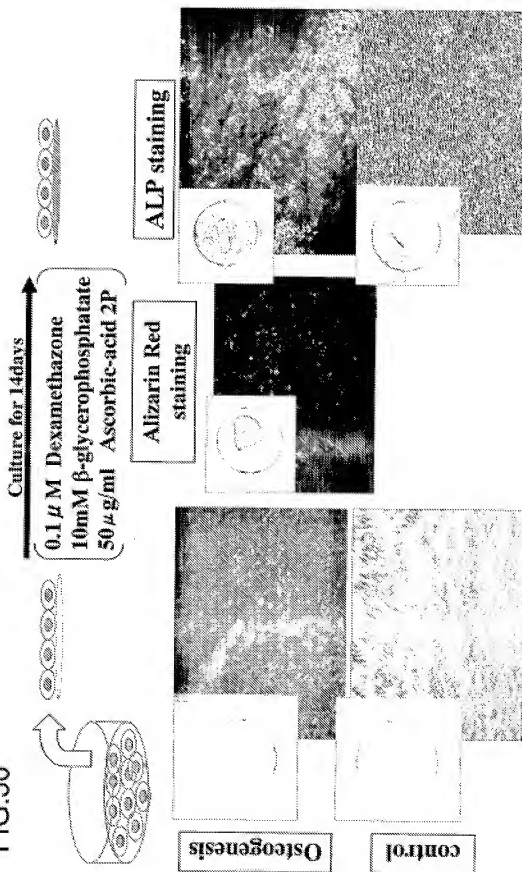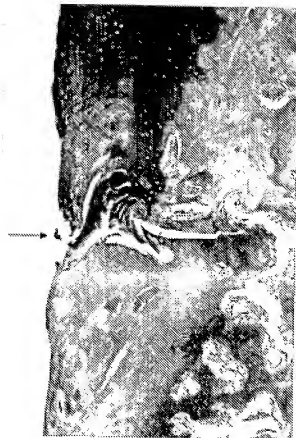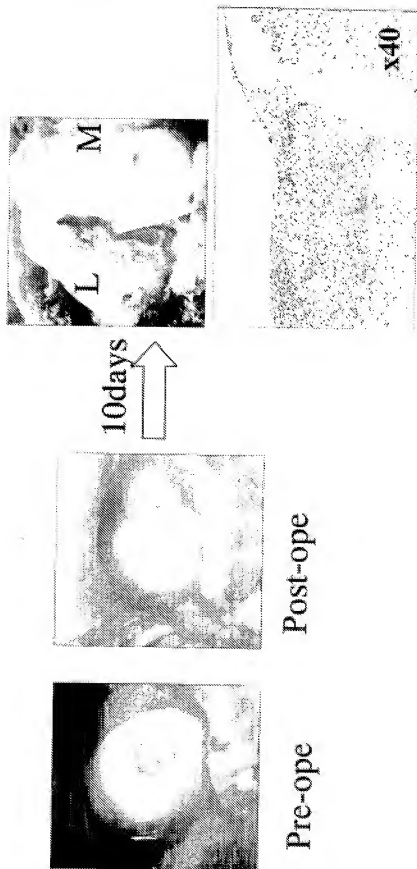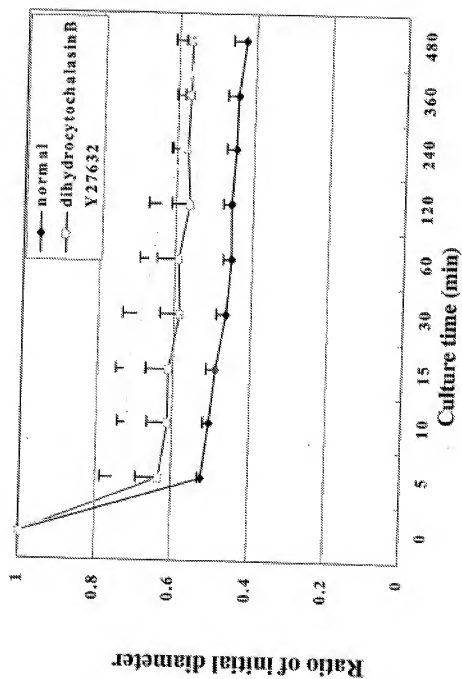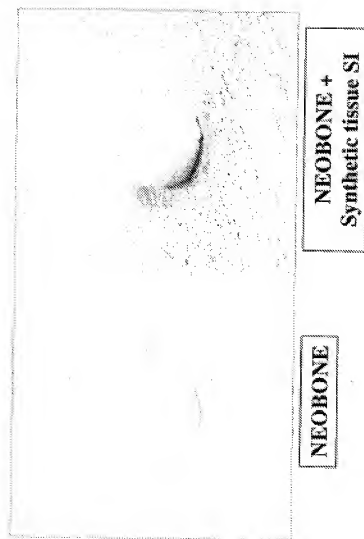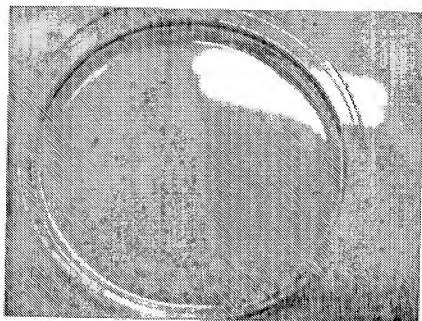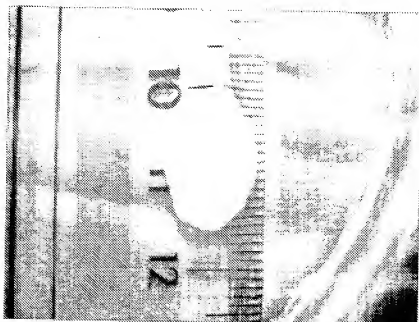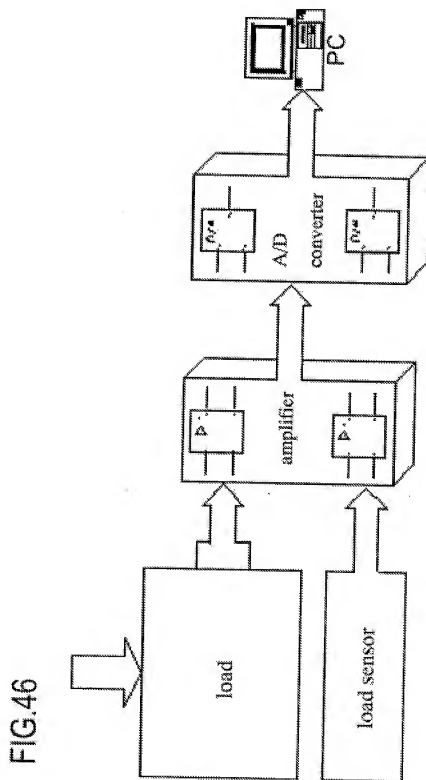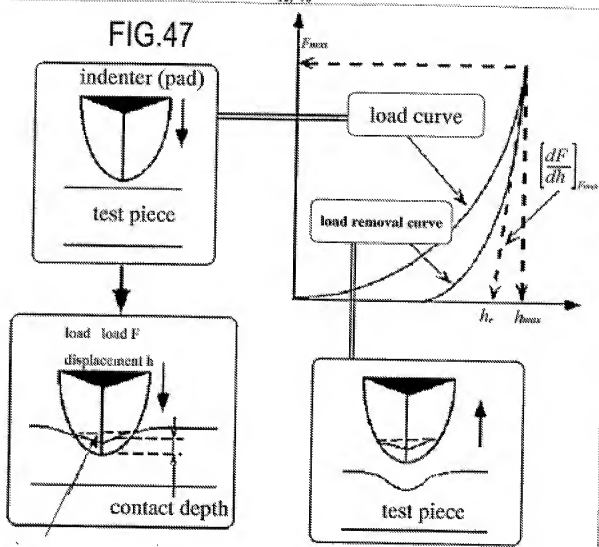
contact
projection A

$$H = \frac{F}{A} = \frac{F}{k_1 h_p^2}$$

$$E = \left[\frac{dF}{dh}\right]_{Fmax} \frac{1-\nu^2}{2 \cdot k_2 \cdot h_{pmax}}$$

$$h_p = h_r + 0.25(h_{max} - h_r)$$

F: load
A: contact projection area
hp: contact depth
$k1k2$: shape conflict
$Fmax$: Maximum load
$hmax$: Maximum displacement
hr: point at which tangential
line intersects
dF/dh: Gradient of tangential
line of load removal curve
ν : Poisson's ratio

SEQUENCE LISTING

<110>   NAKAMURA, Norimasa; MATSUDA, Hikaru; SAWA, Yoshiki; TAKETANI,
Satoshi;MIYAGAWA, Shigeru; YOSHIKAWA, Hideki; ANDO, Wataru

<120>   SCAFFOLD-FREE SELF-ORGANIZED 3D SYNTHETIC TISSUE

<130>   NKM001PCT

<160>   30

<170>   PatentIn version 3.2

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<212>   DNA
<213>   Homo sapiens

<220>
<221>   CDS
<222>   (115)..(5940)

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actccccaggc tacatcttct cacttgctaa caaggaccto tgagttcagc agcc atg   117
                                                                  Met
                                                                    1

agt tca gac tca gaa ttg gct gtt ttt ggg gag gct gct cct ttc ctc   165
Ser Ser Asp Ser Glu Leu Ala Val Phe Gly Glu Ala Ala Pro Phe Leu
            5   10   15

cga aag tct gaa agg gag cgc att gag gcc cag aat agg ccc ttt gat   213
Arg Lys Ser Glu Arg Glu Arg Ile Glu Ala Gln Asn Arg Pro Phe Asp

```
                20                    25                    30

gcc aaa aca tct gtc ttt gtg gcg gag ccc aaa gaa tcc ttt gtc aaa      261
Ala Lys Thr Ser Val Phe Val Ala Glu Pro Lys Glu Ser Phe Val Lys
   35                    40                    45

ggg acc atc cag agc aga gaa gga gga aaa gtg acg gtg aag act gag      309
Gly Thr Ile Gln Ser Arg Glu Gly Gly Lys Val Thr Val Lys Thr Glu
50                    55                    60                    65

gga gga gcg act ctg aca gtg aag gat gat cag gtc ttc ccc atg aac      357
Gly Gly Ala Thr Leu Thr Val Lys Asp Asp Gln Val Phe Pro Met Asn
                70                    75                    80

cct ccc aaa tat gac aag atc gag gat atg gcc atg atg act cat ctg      405
Pro Pro Lys Tyr Asp Lys Ile Glu Asp Met Ala Met Met Thr His Leu
                85                    90                    95

cat gag cct gct gtg ctg tac aac ctc aaa gaa cgt tat gca gcc tgg      453
His Glu Pro Ala Val Leu Tyr Asn Leu Lys Glu Arg Tyr Ala Ala Trp
               100                   105                   110

atg atc tac acc tat tca ggt ctc ttc tgt gtc act gtc aac ccc tac      501
Met Ile Tyr Thr Tyr Ser Gly Leu Phe Cys Val Thr Val Asn Pro Tyr
   115                   120                   125

aag tgg ctg cct gtg tat aag ccc gag gtg gtg aca gcc tac cga ggc      549
Lys Trp Leu Pro Val Tyr Lys Pro Glu Val Val Thr Ala Tyr Arg Gly
130                   135                   140                   145

aaa aag cgc cag ggg gcc ccg ccc cac atc ttc tcc atc tct gac aac      597
Lys Lys Arg Gln Gly Ala Pro Pro His Ile Phe Ser Ile Ser Asp Asn
               150                   155                   160

gcc tat cag ttc atg ctg act gac cga gag aat cag tca atc ctg atc      645
Ala Tyr Gln Phe Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu Ile
```

        165   170   175

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act gga gaa tct ggt gca ggg aag act gtg aac acc aag cgt gtc atc       693
Thr Gly Glu Ser Gly Ala Gly Lys Thr Val Asn Thr Lys Arg Val Ile
        180             185             190

cag tac ttt gca aca att gca gtt act ggt gag aag aag aag gaa gaa       741
Gln Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu Glu
    195             200             205

att act tct ggc aaa ata cag ggg act ctg gaa gat caa atc atc agt       789
Ile Thr Ser Gly Lys Ile Gln Gly Thr Leu Glu Asp Gln Ile Ile Ser
210             215             220             225

gcc aac ccc cta ctg gag gcc ttt ggc aac gcc aag acc gtg agg aat       837
Ala Asn Pro Leu Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg Asn
            230             235             240

gac aac tcc tct cgc ttt ggt aaa ttc atc aga atc cac ttt ggc act       885
Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly Thr
            245             250             255

act gga aaa ctg gca tct gct gat att gaa aca tat ctg cta gag aag       933
Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu Lys
        260             265             270

tct aga gtt gtt ttc cag ctt aag gct gag aga agt tat cat att ttt       981
Ser Arg Val Val Phe Gln Leu Lys Ala Glu Arg Ser Tyr His Ile Phe
        275             280             285

tac cag att aca tcg aat aag aaa cca gaa ctt att gaa atg ctt ctg       1029
Tyr Gln Ile Thr Ser Asn Lys Lys Pro Glu Leu Ile Glu Met Leu Leu
290             295             300             305

att acc acg aac cca tat gat tac cca ttt gtc agt caa ggg gag atc       1077
Ile Thr Thr Asn Pro Tyr Asp Tyr Pro Phe Val Ser Gln Gly Glu Ile
```

```
                    310                315                320

agt gtg gcc agc atc gat gat cag gaa gaa ctg atg gcc aca gat agt     1125
Ser Val Ala Ser Ile Asp Asp Gln Glu Glu Leu Met Ala Thr Asp Ser
           325                330                335

gct att gat att ttg ggc ttt act aat gaa gaa aag gtc tcc att tac     1173
Ala Ile Asp Ile Leu Gly Phe Thr Asn Glu Glu Lys Val Ser Ile Tyr
           340                345                350

aag ctc acg ggg gct gtg atg cat tat ggg aac cta aaa ttt aag caa     1221
Lys Leu Thr Gly Ala Val Met His Tyr Gly Asn Leu Lys Phe Lys Gln
           355                360                365

aag cag cgt gag gag caa gca gag cca gat ggc aca gaa gtt gct gac     1269
Lys Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Val Ala Asp
370                375                380                385

aag gcg gcc tac ctc cag agt ctg aac tct gca gat ctg ctc aaa gct     1317
Lys Ala Ala Tyr Leu Gln Ser Leu Asn Ser Ala Asp Leu Leu Lys Ala
                390                395                400

ctc tgc tac ccc agg gtc aag gtc ggc aat gag tat gtc acc aaa ggc     1365
Leu Cys Tyr Pro Arg Val Lys Val Gly Asn Glu Tyr Val Thr Lys Gly
           405                410                415

cag act gta gaa cag gtg tcc aac gca gta ggt gct ctg gcc aaa gcc     1413
Gln Thr Val Glu Gln Val Ser Asn Ala Val Gly Ala Leu Ala Lys Ala
           420                425                430

gtc tac gag aag atg ttc ctg tgg atg gtt gcc cgc atc aac cag cag     1461
Val Tyr Glu Lys Met Phe Leu Trp Met Val Ala Arg Ile Asn Gln Gln
           435                440                445

ctg gac acc aag cag ccc agg cag tac ttc atc ggg gtc ttg gac att     1509
Leu Asp Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp Ile
```

```
        450             455              460              465

gct ggt ttt gag att ttt gat ttc aac agc ctg gag cag ctg tgc atc       1557
Ala Gly Phe Glu Ile Phe Asp Phe Asn Ser Leu Glu Gln Leu Cys Ile
                470              475              480

aac ttc acc aat gag aaa ctg caa cag ttt ttc aac cac cac atg ttc       1605
Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met Phe
             485              490              495

gtg ctg gag cag gag gag tac aag aag gaa ggc atc gag tgg acg ttc       1653
Val Leu Glu Gln Glu Glu Tyr Lys Lys Glu Gly Ile Glu Trp Thr Phe
          500              505              510

atc gac ttc ggg atg gac ctg gct gcc tgc atc gag ctc atc gag aag       1701
Ile Asp Phe Gly Met Asp Leu Ala Ala Cys Ile Glu Leu Ile Glu Lys
       515              520              525

cct atg ggc atc ttc tcc atc ctg gaa gag gag tgc atg ttc cct aag       1749
Pro Met Gly Ile Phe Ser Ile Leu Glu Glu Glu Cys Met Phe Pro Lys
530              535              540              545

gca aca gac acc tcc ttc aag aac aag ctg tat gac cag cac ctg ggc       1797
Ala Thr Asp Thr Ser Phe Lys Asn Lys Leu Tyr Asp Gln His Leu Gly
             550              555              560

aag tct gcc aac ttc cag aag ccc aag gtg gtc aaa ggc aag gcc gag       1845
Lys Ser Ala Asn Phe Gln Lys Pro Lys Val Val Lys Gly Lys Ala Glu
          565              570              575

gcc cac ttc gct ctg att cac tat gct ggt gtt gtg gac tac aac att       1893
Ala His Phe Ala Leu Ile His Tyr Ala Gly Val Val Asp Tyr Asn Ile
       580              585              590

act ggc tgg ctg gag aag aac aag gac ccc ctg aat gag acc gtg gtt       1941
Thr Gly Trp Leu Glu Lys Asn Lys Asp Pro Leu Asn Glu Thr Val Val
```

```
        595                   600                   605

gga ctg tac cag aag tct gca atg aaa act cta gct cag ctc ttc tct   1989
Gly Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Gln Leu Phe Ser
610                   615                   620                   625

ggg gct caa act gct gaa gga gag gga gct ggc gga ggg gca aag aaa   2037
Gly Ala Gln Thr Ala Glu Gly Glu Gly Ala Gly Gly Gly Ala Lys Lys
                  630                   635                   640

ggt ggt aag aag aag ggc tct tct ttc cag aca gtg tct gcc ctt ttc   2085
Gly Gly Lys Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu Phe
                  645                   650                   655

aga gag aat ttg aac aag ctg atg acc aac ctc agg agt acc cat cct   2133
Arg Glu Asn Leu Asn Lys Leu Met Thr Asn Leu Arg Ser Thr His Pro
             660                   665                   670

cac ttt gtg agg tgt atc atc ccc aat gag aca aaa act cct ggt gcc   2181
His Phe Val Arg Cys Ile Ile Pro Asn Glu Thr Lys Thr Pro Gly Ala
        675                   680                   685

atg gag cat gag ctt gtc ctc cac cag ctg agg tgt aac ggt gtg ctg   2229
Met Glu His Glu Leu Val Leu His Gln Leu Arg Cys Asn Gly Val Leu
690                   695                   700                   705

gaa ggc atc cgc atc tgt agg aaa gga ttt ccc agc aga atc ctt tat   2277
Glu Gly Ile Arg Ile Cys Arg Lys Gly Phe Pro Ser Arg Ile Leu Tyr
                  710                   715                   720

gca gac ttc aaa cag aga tac aag gta tta aat gca agt gca atc cct   2325
Ala Asp Phe Lys Gln Arg Tyr Lys Val Leu Asn Ala Ser Ala Ile Pro
             725                   730                   735

gaa ggg caa ttc att gat agc aag aag gcc tct gag aag ctc ctt gca   2373
Glu Gly Gln Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu Ala
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                740                 745                 750

tcc atc gac att gac cac acc cag tat aaa ttt ggg cac acc aag gtc    2421
Ser Ile Asp Ile Asp His Thr Gln Tyr Lys Phe Gly His Thr Lys Val
    755                 760                 765

ttt ttc aaa gct ggt ctt ctg ggg ctc cta gag gag atg cga gat gac    2469
Phe Phe Lys Ala Gly Leu Leu Gly Leu Leu Glu Glu Met Arg Asp Asp
770                 775                 780                 785

aag ctg gcc cag ctg att acc cga acc cag gcc agg tgc aga ggg ttc    2517
Lys Leu Ala Gln Leu Ile Thr Arg Thr Gln Ala Arg Cys Arg Gly Phe
                790                 795                 800

ttg gca aga gtg gag tac cag agg atg gtg gag aga agg gag gcc atc    2565
Leu Ala Arg Val Glu Tyr Gln Arg Met Val Glu Arg Arg Glu Ala Ile
                805                 810                 815

ttc tgt atc cag tac aat atc aga tcc ttc atg aat gtc aag cac tgg    2613
Phe Cys Ile Gln Tyr Asn Ile Arg Ser Phe Met Asn Val Lys His Trp
        820                 825                 830

ccc tgg atg aaa ctc ttc ttc aag atc aag cct ctg ttg aag agt gca    2661
Pro Trp Met Lys Leu Phe Phe Lys Ile Lys Pro Leu Leu Lys Ser Ala
        835                 840                 845

gaa act gag aag gag atg gcc acc atg aag gaa gaa ttt cag aaa att    2709
Glu Thr Glu Lys Glu Met Ala Thr Met Lys Glu Glu Phe Gln Lys Ile
850                 855                 860                 865

aaa gac gaa ctt gcc aag tca gag gca aaa agg aag gaa ctg gag gaa    2757
Lys Asp Glu Leu Ala Lys Ser Glu Ala Lys Arg Lys Glu Leu Glu Glu
                870                 875                 880

aag atg gtg acg ctg ttg aaa gaa aaa aat gac ttg cag ctc caa gtt    2805
Lys Met Val Thr Leu Leu Lys Glu Lys Asn Asp Leu Gln Leu Gln Val
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              885                 890                 895

cag gct gaa goc gaa ggc ttg gct gat gca gag gaa agg tgt gac cag     2853
Gln Ala Glu Ala Glu Gly Leu Ala Asp Ala Glu Glu Arg Cys Asp Gln
         900                 905                 910

cta atc aaa acc aaa atc cag cta gaa gcc aaa atc aaa gag gtg act     2901
Leu Ile Lys Thr Lys Ile Gln Leu Glu Ala Lys Ile Lys Glu Val Thr
     915                 920                 925

gag aga gct gag gat gag gaa gag atc aat gct gag ctg aca gcc aag     2949
Glu Arg Ala Glu Asp Glu Glu Glu Ile Asn Ala Glu Leu Thr Ala Lys
930                 935                 940                 945

aag agg aaa ctg gag gat gaa tgt tca gaa ctc aag aaa gac att gat     2997
Lys Arg Lys Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile Asp

gac ctt gag ctg aca ctg gcc aag gtt gag aag gag aaa cat gcc aca     3045
Asp Leu Glu Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala Thr
         965                 970                 975

gaa aac aag gtg aaa aac ctc aca gaa gag atg gca ggt ctg gat gaa     3093
Glu Asn Lys Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp Glu
         980                 985                 990

acc att gct aag ctg acc aag  gag aag aag gct ctc  cag gag gcc cac    3141
Thr Ile Ala Lys Leu Thr Lys  Glu Lys Lys Ala Leu  Gln Glu Ala His
     995                 1000                1005

cag  cag acc ctg gat gac  ctg cag gca gag gag  gac aaa gtc aac       3186
Gln  Gln Thr Leu Asp Asp  Leu Gln Ala Glu Glu  Asp Lys Val Asn
1010                1015                1020

acc  ctg acc aaa gct aaa  atc aaa ctt gaa caa  caa gtg gat gat       3231
Thr  Leu Thr Lys Ala Lys  Ile Lys Leu Glu Gln  Gln Val Asp Asp
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        1025              1030              1035

ctt  gaa gag tcc ttg gag  caa gaa aag aaa ctt  cgc atg gac cta    3276
Leu  Glu Gly Ser Leu Glu  Gln Glu Lys Lys Leu  Arg Met Asp Leu
1040              1045              1050


gaa  agg gct aag agg aaa  ctt gag gat gac ttg  aag ttg gcc caa    3321
Glu  Arg Ala Lys Arg Lys  Leu Glu Gly Asp Leu  Lys Leu Ala Gln
1055              1060              1065


gaa  tcc ata atg gac att  gaa aat gag aaa cag  caa ctt gat gaa    3366
Glu  Ser Ile Met Asp Ile  Glu Asn Glu Lys Gln  Gln Leu Asp Glu
1070              1075              1080


aag  ctc aaa aag aaa gag  ttt gaa atc agc aat  ctg caa agc aag    3411
Lys  Leu Lys Lys Lys Glu  Phe Glu Ile Ser Asn  Leu Gln Ser Lys
1085              1090              1095


att  gaa gat gaa cag gca  ctt ggc att caa ttg  cag aag aaa att    3456
Ile  Glu Asp Glu Gln Ala  Leu Gly Ile Gln Leu  Gln Lys Lys Ile
1100              1105              1110


aaa  gaa ttg caa gcc cgc  att gag gag ctg gag  gag gaa atc gag    3501
Lys  Glu Leu Gln Ala Arg  Ile Glu Glu Leu Glu  Glu Glu Ile Glu
1115              1120              1125


gca  gag cgg gcc tcc cgg  gcc aaa gca gag aag  cag cgc tct gac    3546
Ala  Glu Arg Ala Ser Arg  Ala Lys Ala Glu Lys  Gln Arg Ser Asp
1130              1135              1140


ctc  tcc cgg gag ctg gag  gag atc agc gag agg  ctg gaa gaa gcc    3591
Leu  Ser Arg Glu Leu Glu  Glu Ile Ser Glu Arg  Leu Glu Glu Ala
1145              1150              1155


ggt  ggg gcc act tca gcc  cag att gag atg aac  aag aag cgg gag    3636
Gly  Gly Ala Thr Ser Ala  Gln Ile Glu Met Asn  Lys Lys Arg Glu
```

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1160                    1166                    1170

gct  gag ttc cag aaa atg  cgc agg gac ctg gag  gag gcc acc cta      3681
Ala  Glu Phe Gln Lys Met  Arg Arg Asp Leu Glu  Glu Ala Thr Leu
1175                    1180                    1185

cag  cat gaa gcc aca gcg  gcc acc ctg agg aag  aag cat gca gat      3726
Gln  His Glu Ala Thr Ala  Ala Thr Leu Arg Lys  Lys His Ala Asp
1190                    1195                    1200

agt  gtg gcc gag ctt ggg  gag cag att gac aac  ctg cag cga gtg      3771
Ser  Val Ala Glu Leu Gly  Glu Gln Ile Asp Asn  Leu Gln Arg Val
1205                    1210                    1215

aag  cag aag ctg gag aag  gag aag agt gag atg  aag atg gag att      3816
Lys  Gln Lys Leu Glu Lys  Glu Lys Ser Glu Met  Lys Met Glu Ile
1220                    1225                    1230

gat  gac ctt gct agt aat  gta gaa acg gtc tcc  aaa gcc aag gga      3861
Asp  Asp Leu Ala Ser Asn  Val Glu Thr Val Ser  Lys Ala Lys Gly
1235                    1240                    1245

aac  cta gag aaa atg tgc  cgg act cta gag gac  caa ctg agt gaa      3906
Asn  Leu Glu Lys Met Cys  Arg Thr Leu Glu Asp  Gln Leu Ser Glu
1250                    1255                    1260

ctg  aaa tca aag gaa gag  gag cag cag cgg ctg  atc aat gac ctg      3951
Leu  Lys Ser Lys Glu Glu  Glu Gln Gln Arg Leu  Ile Asn Asp Leu
1265                    1270                    1275

act  gcg cag agg ggg cgc  ctg cag act gaa tct  ggt gag ttt tca      3996
Thr  Ala Gln Arg Gly Arg  Leu Gln Thr Glu Ser  Gly Glu Phe Ser
1280                    1285                    1290

cgc  cag ctt gat gaa aag  gaa gct ctg gtg tct  cag tta tca aga      4041
Arg  Gln Leu Asp Glu Lys  Glu Ala Leu Val Ser  Gln Leu Ser Arg
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        1295              1300              1305

ggc  aaa caa gcc ttt act  caa cag att gaa gaa  tta aag agg caa     4086
Gly  Lys Gln Ala Phe Thr  Gln Gln Ile Glu Glu  Leu Lys Arg Gln
1310              1315              1320

ctt  gaa gag gag ata aaa  gcc aag aac gcc ctg  gcg aat gcc ctg     4131
Leu  Glu Glu Glu Ile Lys  Ala Lys Asn Ala Leu  Ala His Ala Leu
1325              1330              1335

cag  tct tcc cgc cac gac  tgt gac ctg ctg cgg  gaa cag tat gag     4176
Gln  Ser Ser Arg His Asp  Cys Asp Leu Leu Arg  Glu Gln Tyr Glu
1340              1345              1350

gag  gag cag gaa tcc aag  gcc gag ctg cag aga  gca ctg tcc aag     4221
Glu  Glu Gln Glu Ser Lys  Ala Glu Leu Gln Arg  Ala Leu Ser Lys
1355              1360              1365

gcc  aac acc gag gtt gcc  caa tgg agg acc aaa  tac gag acg gac     4266
Ala  Asn Thr Glu Val Ala  Gln Trp Arg Thr Lys  Tyr Glu Thr Asp
1370              1375              1380

gcc  atc cag cgc aca gag  gag ctg gag gag gcc  aag aag aag ctg     4311
Ala  Ile Gln Arg Thr Glu  Glu Leu Glu Glu Ala  Lys Lys Lys Leu
1385              1390              1395

gcc  cag cgg ctg cag gca  gct gag gaa cat gta  gaa gct gtg aac     4356
Ala  Gln Arg Leu Gln Ala  Ala Glu Glu His Val  Glu Ala Val Asn
1400              1405              1410

gcc  aaa tgt gct tcc ctc  gaa aag acg aag cag  cgg ctg cag aat     4401
Ala  Lys Cys Ala Ser Leu  Glu Lys Thr Lys Gln  Arg Leu Gln Asn
1415              1420              1425

gag  gtc gag gac ctc atg  ctt gat gtg gag agg  aca aat gcc gcc     4446
Glu  Val Glu Asp Leu Met  Leu Asp Val Glu Arg  Thr Asn Ala Ala
```

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      1430              1435              1440

tgt  gcc gcc ctt gac aaa  aag caa agg aac ttc  gat aag atc ctg    4491
Cys  Ala Ala Leu Asp Lys  Lys Gln Arg Asn Phe  Asp Lys Ile Leu
1445              1450              1455

gca  gaa tgg aaa cag aaa  tgt gag gaa acg cat  gct gag ctt gag    4536
Ala  Glu Trp Lys Gln Lys  Cys Glu Glu Thr His  Ala Glu Leu Glu
1460              1465              1470

gcc  tcc cag aag gag gcc  cgt tcc ctt ggc act  gag ctg ttc aag    4581
Ala  Ser Gln Lys Glu Ala  Arg Ser Leu Gly Thr  Glu Leu Phe Lys
1475              1480              1485

ata  aag aat gcc tat gag  gaa tct ttg gat cag  cta gaa acc ctg    4626
Ile  Lys Asn Ala Tyr Glu  Glu Ser Leu Asp Gln  Leu Glu Thr Leu
1490              1495              1500

aag  cga gag aac aaa aac  tta cag cag gag att  tct gac ctc acg    4671
Lys  Arg Glu Asn Lys Asn  Leu Gln Gln Glu Ile  Ser Asp Leu Thr
1505              1510              1515

gaa  cag att gca gaa gga  ggg aaa cgt atc cat  gaa ctg gag aaa    4716
Glu  Gln Ile Ala Glu Gly  Gly Lys Arg Ile His  Glu Leu Glu Lys
1520              1525              1530

ata  aag aaa caa gtg gaa  caa gaa aag tgt gaa  ctt cag gct gct    4761
Ile  Lys Lys Gln Val Glu  Gln Glu Lys Cys Glu  Leu Gln Ala Ala
1535              1540              1545

tta  gaa gaa gca gag gca  tct ctt gaa cat gaa  gag gga aag atc    4806
Leu  Glu Glu Ala Glu Ala  Ser Leu Glu His Glu  Glu Gly Lys Ile
1550              1555              1560

ctg  cgc atc cag ctt gag  ttg aac cag gtc aag  tct gag gtt gat    4851
Leu  Arg Ile Gln Leu Glu  Leu Asn Gln Val Lys  Ser Glu Val Asp
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    1565                 1570                 1575

    agg  aaa att gct gaa aaa  gat gag gaa att gac  cag ctg aag aga    4896
    Arg  Lys Ile Ala Glu Lys  Asp Glu Glu Ile Asp  Gln Leu Lys Arg
    1580                 1585                 1590

    aac  cac att aga atc gtg  gag tcc atg cag agc  acg ctg gat gct    4941
    Asn  His Ile Arg Ile Val  Glu Ser Met Gln Ser  Thr Leu Asp Ala
    1595                 1600                 1605

    gag  atc agg agt agg aat  gat gcc att agg ctc  aag aag aag atg    4986
    Glu  Ile Arg Ser Arg Asn  Asp Ala Ile Arg Leu  Lys Lys Lys Met
    1610                 1615                 1620

    gag  gga gac ctc aat gaa  atg gaa atc cag ctg  aac cat gcc aac    5031
    Glu  Gly Asp Leu Asn Glu  Met Glu Ile Gln Leu  Asn His Ala Asn
    1625                 1630                 1635

    cgg  atg gct gct gag gcc  ctg agg aac tac agg  aac acc caa ggc    5076
    Arg  Met Ala Ala Glu Ala  Leu Arg Asn Tyr Arg  Asn Thr Gln Gly
    1640                 1645                 1650

    atc  ctc aag gat acc cag  atc cac ctg gat gat  gct ctc cgg agc    5121
    Ile  Leu Lys Asp Thr Gln  Ile His Leu Asp Asp  Ala Leu Arg Ser
    1655                 1660                 1665

    cag  gag gac ctg aag gaa  cag ctg gcc atg gtg  gag cgc aga gcc    5166
    Gln  Glu Asp Leu Lys Glu  Gln Leu Ala Met Val  Glu Arg Arg Ala
    1670                 1675                 1680

    aac  ctg ctg cag gct gag  atc gag gag ctg cgg  gcc act ctg gaa    5211
    Asn  Leu Leu Gln Ala Glu  Ile Glu Glu Leu Arg  Ala Thr Leu Glu
    1685                 1690                 1695

    cag  aca gag agg agc aga  aaa atc gca gaa cag  gag ctc ctg gat    5256
    Gln  Thr Glu Arg Ser Arg  Lys Ile Ala Glu Gln  Glu Leu Leu Asp
```

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            1700              1705              1710

gcc  agt gag cgt gtt cag  cta ctg cac acc cag  aac acc agc ctg      5301
Ala  Ser Glu Arg Val Gln  Leu Leu His Thr Gln  Asn Thr Ser Leu
1715              1720              1725

atc  aac acc aag aag aag  ctg gag aca gat att  tcc caa atg caa      5346
Ile  Asn Thr Lys Lys Lys  Leu Glu Thr Asp Ile  Ser Gln Met Gln
1730              1735              1740

gga  gag atg gag gac att  ctc cag gaa gcc cgc  aat gca gaa gaa      5391
Gly  Glu Met Glu Asp Ile  Leu Gln Glu Ala Arg  Asn Ala Glu Glu
1745              1750              1755

aag  gcc aag aag gcc atc  act gat gcc gcc atg  atg gct gag gag      5436
Lys  Ala Lys Lys Ala Ile  Thr Asp Ala Ala Met  Met Ala Glu Glu
1760              1765              1770

ctg  aag aag gag cag gac  acc agc gcc cac ctg  gag cgg atg aag      5481
Leu  Lys Lys Glu Gln Asp  Thr Ser Ala His Leu  Glu Arg Met Lys
1775              1780              1785

aag  aac atg gag cag acc  gtg aag gat ctg cag  ctc cgt ctg gat      5526
Lys  Asn Met Glu Gln Thr  Val Lys Asp Leu Gln  Leu Arg Leu Asp
1790              1795              1800

gag  gct gag cag ctg gcc  ctg aag ggt ggg aag  aag cag atc cag      5571
Glu  Ala Glu Gln Leu Ala  Leu Lys Gly Gly Lys  Lys Gln Ile Gln
1805              1810              1815

aaa  ctg gag gcc agg gta  cgg gag ctg gaa gga  gag gtt gag agt      5616
Lys  Leu Glu Ala Arg Val  Arg Glu Leu Glu Gly  Glu Val Glu Ser
1820              1825              1830

gag  caa aag cgt aat gct  gag gct gtc aaa ggt  ctc cgc aaa cat      5661
Glu  Gln Lys Arg Asn Ala  Glu Ala Val Lys Gly  Leu Arg Lys His
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      1835                  1840                  1845

      gag  agg cga gtg aag gaa  ctc act tac cag acg  gaa gaa gat aga        5706
      Glu  Arg Arg Val Lys Glu  Leu Thr Tyr Gln Thr  Glu Glu Asp Arg
      1850                 1855                 1860

      aag  aat att ctc agg ctt  caa gat ttg gta gat  aaa ctt cag gca        5751
      Lys  Asn Ile Leu Arg Leu  Gln Asp Leu Val Asp  Lys Leu Gln Ala
      1865                 1870                 1875

      aaa  gtg aaa tct tat aag  aga caa gct gag gag  gct gag gaa caa        5796
      Lys  Val Lys Ser Tyr Lys  Arg Gln Ala Glu Glu  Ala Glu Glu Gln
      1880                 1885                 1890

      tcc  aac acc aat cta gct  aaa ttc cgc aag ctc  cag cat gag ctg        5841
      Ser  Asn Thr Asn Leu Ala  Lys Phe Arg Lys Leu  Gln His Glu Leu
      1895                 1900                 1905

      gag  gag gcc gag gaa cgg  gct gac att gct gag  tcc cag gtg aac        5886
      Glu  Glu Ala Glu Glu Arg  Ala Asp Ile Ala Glu  Ser Gln Val Asn
      1910                 1915                 1920

      aaa  ctg cgg gtg aag agc  cgg gag gtt cac aca  aaa gtc ata agt        5931
      Lys  Leu Arg Val Lys Ser  Arg Glu Val His Thr  Lys Val Ile Ser
      1925                 1930                 1935

      gaa  gag tga tcatgtcctg atgccatgga atgactgaag acaggcacaa             5980
      Glu  Glu
      1940

      aatgtgacat cttggtcat ttccctctgt aattattgtg tattctaccc tgttgcaaag     6040

      gaaatasagc ataggtagt ttgcaaacaa aaaaaaaaaa aaaaa                     6085


      <210>  2
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<211> 1941
<212> PRT
<213> Homo sapiens

<400> 2

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Leu Arg Lys Ser Glu Arg Glu Arg Ile Glu Ala Gln Asn Arg Pro Phe
            20   25   30

Asp Ala Lys Thr Ser Val Phe Val Ala Glu Pro Lys Glu Ser Phe Val
        35   40   45

Lys Gly Thr Ile Gln Ser Arg Glu Gly Gly Lys Val Thr Val Lys Thr
    50   55   60

Glu Gly Gly Ala Thr Leu Thr Val Lys Asp Asp Gln Val Phe Pro Met
65   70   75   80

Asn Pro Pro Lys Tyr Asp Lys Ile Glu Asp Met Ala Met Met Thr His
            85   90   95

Leu His Glu Pro Ala Val Leu Tyr Asn Leu Lys Glu Arg Tyr Ala Ala
            100   105   110

Trp Met Ile Tyr Thr Tyr Ser Gly Leu Phe Cys Val Thr Val Asn Pro
        115   120   125

Tyr Lys Trp Leu Pro Val Tyr Lys Pro Glu Val Val Thr Ala Tyr Arg
        130   135   140

Gly Lys Lys Arg Gln Gly Ala Pro Pro His Ile Phe Ser Ile Ser Asp
145   150   155   160

Asn Ala Tyr Gln Phe Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu
                165   170   175

Ile Thr Gly Glu Ser Gly Ala Gly Lys Thr Val Asn Thr Lys Arg Val
                180   185   190

Ile Gln Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu
        195   200   205

Glu Ile Thr Ser Gly Lys Ile Gln Gly Thr Leu Glu Asp Gln Ile Ile
        210   215   220

Ser Ala Asn Pro Leu Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg
225   230   235   240

Asn Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly
                245   250   255

Thr Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu
                260   265   270

Lys Ser Arg Val Val Phe Gln Leu Lys Ala Glu Arg Ser Tyr His Ile
        275   280   285

Phe Tyr Gln Ile Thr Ser Asn Lys Lys Pro Glu Leu Ile Glu Met Leu
    290   295   300

Leu Ile Thr Thr Asn Pro Tyr Asp Tyr Pro Phe Val Ser Gln Gly Glu
305   310   315   320

Ile Ser Val Ala Ser Ile Asp Asp Gln Glu Glu Leu Met Ala Thr Asp
            325   330   335

Ser Ala Ile Asp Ile Leu Gly Phe Thr Asn Glu Glu Lys Val Ser Ile
            340   345   350

Tyr Lys Leu Thr Gly Ala Val Met His Tyr Gly Asn Leu Lys Phe Lys
        355   360   365

Gln Lys Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Val Ala
    370   375   380

Asp Lys Ala Ala Tyr Leu Gln Ser Leu Asn Ser Ala Asp Leu Leu Lys
385   390   395   400

Ala Leu Cys Tyr Pro Arg Val Lys Val Gly Asn Glu Tyr Val Thr Lys
            405   410   415

Gly Gln Thr Val Glu Gln Val Ser Asn Ala Val Gly Ala Leu Ala Lys
        420   425   430

Ala Val Tyr Glu Lys Met Phe Leu Trp Met Val Ala Arg Ile Asn Gln
      435   440   445

Gln Leu Asp Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp
  450   455   460

Ile Ala Gly Phe Glu Ile Phe Asp Phe Asn Ser Leu Glu Gln Leu Cys
 465   470   475   480

Ile Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met
        485   490   495

Phe Val Leu Glu Gln Glu Glu Tyr Lys Lys Glu Gly Ile Glu Trp Thr
        500   505   510

Phe Ile Asp Phe Gly Met Asp Leu Ala Ala Cys Ile Glu Leu Ile Glu
      515   520   525

Lys Pro Met Gly Ile Phe Ser Ile Leu Glu Glu Glu Cys Met Phe Pro
    530   535   540

Lys Ala Thr Asp Thr Ser Phe Lys Asn Lys Leu Tyr Asp Gln His Leu
 545   550   555   560

Gly Lys Ser Ala Asn Phe Gln Lys Pro Lys Val Val Lys Gly Lys Ala
              555   570   575

Glu Ala His Phe Ala Leu Ile His Tyr Ala Gly Val Val Asp Tyr Asn
              560   585   590

Ile Thr Gly Trp Leu Glu Lys Asn Lys Asp Pro Leu Asn Glu Thr Val
         595   600   605

Val Gly Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Gln Leu Phe
     610   615   620

Ser Gly Ala Gln Thr Ala Glu Gly Glu Gly Ala Gly Gly Gly Ala Lys
625   630   635   640

Lys Gly Gly Lys Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu
              645   650   655

Phe Arg Glu Asn Leu Asn Lys Leu Met Thr Asn Leu Arg Ser Thr His
              660   665   670

Pro His Phe Val Arg Cys Ile Ile Pro Asn Glu Thr Lys Thr Pro Gly
         675   680   685

Ala Met Glu His Glu Leu Val Leu His Gln Leu Arg Cys Asn Gly Val
     690   695   700

Leu Glu Gly Ile Arg Ile Cys Arg Lys Gly Phe Pro Ser Arg Ile Leu
705   710   715   720

Tyr Ala Asp Phe Lys Gln Arg Tyr Lys Val Leu Asn Ala Ser Ala Ile
                725   730   735

Pro Glu Gly Gln Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu
            740   745   750

Ala Ser Ile Asp Ile Asp His Thr Gln Tyr Lys Phe Gly His Thr Lys
            755   760   765

Val Phe Phe Lys Ala Gly Leu Leu Gly Leu Leu Glu Glu Met Arg Asp
    770   775   780

Asp Lys Leu Ala Gln Leu Ile Thr Arg Thr Gln Ala Arg Cys Arg Gly
785   790   795   800

Phe Leu Ala Arg Val Glu Tyr Gln Arg Met Val Glu Arg Arg Glu Ala
            805   810   815

Ile Phe Cys Ile Gln Tyr Asn Ile Arg Ser Phe Met Asn Val Lys His
            820   825   830

Trp Pro Trp Met Lys Leu Phe Phe Lys Ile Lys Pro Leu Leu Lys Ser
            835   840   845

Ala Glu Thr Glu Lys Glu Met Ala Thr Met Lys Glu Glu Phe Gln Lys
　　　850　　　　　　　　　　855　　　　　　　　　860

Ile Lys Asp Glu Leu Ala Lys Ser Glu Ala Lys Arg Lys Glu Leu Glu
865　　　　　　　　870　　　　　　　875　　　　　　　　880

Glu Lys Met Val Thr Leu Leu Lys Glu Lys Asn Asp Leu Gln Leu Gln
　　　　　　　885　　　　　　　　890　　　　　　　　895

Val Gln Ala Glu Ala Glu Gly Leu Ala Asp Ala Glu Glu Arg Cys Asp
　　　　900　　　　　　　　905　　　　　　　　910

Gln Leu Ile Lys Thr Lys Ile Gln Leu Glu Ala Lys Ile Lys Glu Val
　　　915　　　　　　　　920　　　　　　　　925

Thr Glu Arg Ala Glu Asp Glu Glu Glu Ile Asn Ala Glu Leu Thr Ala
　　　930　　　　　　　　935　　　　　　　940

Lys Lys Arg Lys Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile
945　　　　　　　　950　　　　　　　955　　　　　　　　960

Asp Asp Leu Glu Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala
　　　　　　　965　　　　　　　970　　　　　　　　975

Thr Glu Asn Lys Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp
　　　　980　　　　　　　　985　　　　　　　　990

Glu Thr Ile Ala Lys Leu Thr Lys Glu Lys Lys Ala Leu Gln Glu Ala
          995   1000   1005

His Gln Gln Thr Leu Asp Asp Leu Gln Ala Glu Glu Asp Lys Val
     1010   1015   1020

Asn Thr Leu Thr Lys Ala Lys Ile Lys Leu Glu Gln Gln Val Asp
     1025   1030   1035

Asp Leu Glu Gly Ser Leu Glu Gln Glu Lys Lys Leu Arg Met Asp
     1040   1045   1050

Leu Glu Arg Ala Lys Arg Lys Leu Glu Gly Asp Leu Lys Leu Ala
     1055   1060   1065

Gln Glu Ser Ile Met Asp Ile Glu Asn Glu Lys Gln Gln Leu Asp
     1070   1075   1080

Glu Lys Leu Lys Lys Lys Glu Phe Glu Ile Ser Asn Leu Gln Ser
     1085   1090   1095

Lys Ile Glu Asp Glu Gln Ala Leu Gly Ile Gln Leu Gln Lys Lys
     1100   1105   1110

Ile Lys Glu Leu Gln Ala Arg Ile Glu Glu Leu Glu Glu Glu Ile
     1115   1120   1125

Glu Ala Glu Arg Ala Ser Arg Ala Lys Ala Glu Lys Glu Arg Ser
    1130   1135   1140

Asp Leu Ser Arg Glu Leu Glu Glu Ile Ser Glu Arg Leu Glu Glu
    1145   1150   1155

Ala Gly Gly Ala Thr Ser Ala Gln Ile Glu Met Asn Lys Lys Arg
    1160   1165   1170

Glu Ala Glu Phe Gln Lys Met Arg Arg Asp Leu Glu Glu Ala Thr
    1175   1180   1185

Leu Gln His Glu Ala Thr Ala Ala Thr Leu Arg Lys Lys His Ala
    1190   1195   1200

Asp Ser Val Ala Glu Leu Gly Glu Gln Ile Asp Asn Leu Gln Arg
    1205   1210   1215

Val Lys Gln Lys Leu Glu Lys Glu Lys Ser Glu Met Lys Met Glu
    1220   1225   1230

Ile Asp Asp Leu Ala Ser Asn Val Glu Thr Val Ser Lys Ala Lys
    1235   1240   1245

Gly Asn Leu Glu Lys Met Cys Arg Thr Leu Glu Asp Gln Leu Ser
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Glu Leu Lys Ser Lys Glu Glu Glu Gln Gln Arg Leu Ile Asn Asp
    1265   1270   1275

Leu Thr Ala Gln Arg Gly Arg Leu Gln Thr Glu Ser Gly Glu Phe
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Ser Arg Gln Leu Asp Glu Lys Glu Ala Leu Val Ser Gln Leu Ser
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Arg Gly Lys Gln Ala Phe Thr Gln Gln Ile Glu Glu Leu Lys Arg
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Gln Leu Glu Glu Glu Ile Lys Ala Lys Asn Ala Leu Ala His Ala
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Leu Gln Ser Ser Arg His Asp Cys Asp Leu Leu Arg Glu Gln Tyr
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Glu Glu Glu Gln Glu Ser Lys Ala Glu Leu Gln Arg Ala Leu Ser
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Lys Ala Asn Thr Glu Val Ala Gln Trp Arg Thr Lys Tyr Glu Thr
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Asp Ala Ile Gln Arg Thr Glu Glu Leu Glu Glu Ala Lys Lys Lys
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Leu Ala   Gln Arg Leu Gln Ala   Ala Glu Glu His Val   Glu Ala Val
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Asn Ala   Lys Cys Ala Ser Leu   Glu Lys Thr Lys Gln   Arg Leu Gln
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Asn Glu   Val Glu Asp Leu Met   Leu Asp Val Glu Arg   Thr Asn Ala
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Ala Cys   Ala Ala Leu Asp Lys   Lys Gln Arg Asn Phe   Asp Lys Ile
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Leu Ala   Glu Trp Lys Gln Lys   Cys Glu Glu Thr His   Ala Glu Leu
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Glu Ala   Ser Gln Lys Glu Ala   Arg Ser Leu Gly Thr   Glu Leu Phe
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Lys Ile   Lys Asn Ala Tyr Glu   Glu Ser Leu Asp Gln   Leu Glu Thr
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Leu Lys   Arg Glu Asn Lys Asn   Leu Gln Gln Glu Ile   Ser Asp Leu
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Thr Glu   Gln Ile Ala Glu Gly   Gly Lys Arg Ile His   Glu Leu Glu
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Ala Leu Glu Glu Ala Glu Ala Ser Leu Glu His Glu Glu Gly Lys
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Ile Leu Arg Ile Gln Leu Glu Leu Asn Gln Val Lys Ser Glu Val
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Asp Arg Lys Ile Ala Glu Lys Asp Glu Glu Ile Asp Gln Leu Lys
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Arg Asn His Ile Arg Ile Val Glu Ser Met Gln Ser Thr Leu Asp
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Ala Glu Ile Arg Ser Arg Asn Asp Ala Ile Arg Leu Lys Lys Lys
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Met Glu Gly Asp Leu Asn Glu Met Glu Ile Gln Leu Asn His Ala
    1625   1630   1635

Asn Arg Met Ala Ala Glu Ala Leu Arg Asn Tyr Arg Asn Thr Gln
    1640   1645   1650

Gly Ile Leu Lys Asp Thr Gln Ile His Leu Asp Asp Ala Leu Arg
    1655   1660   1665

Ser Gln Glu Asp Leu Lys Glu Gln Leu Ala Met Val Glu Arg Arg
   1670   1675   1680

Ala Asn Leu Leu Gln Ala Glu Ile Glu Glu Leu Arg Ala Thr Leu
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Glu Gln Thr Glu Arg Ser Arg Lys Ile Ala Glu Gln Glu Leu Leu
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Asp Ala Ser Glu Arg Val Gln Leu Leu His Thr Gln Asn Thr Ser
   1715   1720   1725

Leu Ile Asn Thr Lys Lys Lys Leu Glu Thr Asp Ile Ser Gln Met
   1730   1735   1740

Gln Gly Glu Met Glu Asp Ile Leu Gln Glu Ala Arg Asn Ala Glu
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Glu Lys Ala Lys Lys Ala Ile Thr Asp Ala Ala Met Met Ala Glu
   1760   1765   1770

Glu Leu Lys Lys Glu Gln Asp Thr Ser Ala His Leu Glu Arg Met
   1775   1780   1785

Lys Lys Asn Met Glu Gln Thr Val Lys Asp Leu Gln Leu Arg Leu
   1790   1795   1800

Asp Glu Ala Glu Gln Leu Ala Leu Lys Gly Gly Lys Lys Gln Ile
    1805   1810   1815

Gln Lys Leu Glu Ala Arg Val Arg Glu Leu Glu Gly Glu Val Glu
    1820   1825   1830

Ser Glu Gln Lys Arg Asn Ala Glu Ala Val Lys Gly Leu Arg Lys
    1835   1840   1845

His Glu Arg Arg Val Lys Glu Leu Thr Tyr Gln Thr Glu Glu Asp
    1850   1855   1860

Arg Lys Asn Ile Leu Arg Leu Gln Asp Leu Val Asp Lys Leu Gln
    1865   1870   1875

Ala Lys Val Lys Ser Tyr Lys Arg Gln Ala Glu Glu Ala Glu Glu
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Gln Ser Asn Thr Asn Leu Ala Lys Phe Arg Lys Leu Gln His Glu
    1895   1900   1905

Leu Glu Glu Ala Glu Glu Arg Ala Asp Ile Ala Glu Ser Gln Val
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Ser Glu Glu
     1940

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                                                         Met Ser
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Ser Asp Ser Glu Met Ala Ile Phe Gly Glu Ala Ala Pro Phe Leu Arg
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aag tct gaa aag gag cga att gaa gct cag aac aag cct ttt gat gcc   213
Lys Ser Glu Lys Glu Arg Ile Glu Ala Gln Asn Lys Pro Phe Asp Ala
     20   25   30

aag aca tca gtc ttt gtg gtg gac cct aag gag tcc tac gtg aaa gca   261
Lys Thr Ser Val Phe Val Val Asp Pro Lys Glu Ser Tyr Val Lys Ala
35   40   45   50

ata gtg cag agc agg gaa ggg ggg aag gtg aca gcc aag acc gaa ggt   309

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Ile Val Gln Ser Arg Glu Gly Gly Lys Val Thr Ala Lys Thr Glu Ala
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gga gct act gta act gtg aaa gag gac caa gtc ttc tcc atg aac cct      357
Gly Ala Thr Val Thr Val Lys Glu Asp Gln Val Phe Ser Met Asn Pro
             70              75              80

ccc aaa tat gac aag atc gag gac atg gcc atg atg act cac ctg cat      405
Pro Lys Tyr Asp Lys Ile Glu Asp Met Ala Met Met Thr His Leu His
         85              90              95

gag cct gct gtg ctg tat aac ctc aaa gag cgt tac gca gcc tgg atg      453
Glu Pro Ala Val Leu Tyr Asn Leu Lys Glu Arg Tyr Ala Ala Trp Met
    100             105             110

atc tac acc tac tcg ggc ctc ttc tgt gtc acc gtc aac ccc tac aag   ·  · 501
Ile Tyr Thr Tyr Ser Gly Leu Phe Cys Val Thr Val Asn Pro Tyr Lys
115             120             125             130

tgg ctg ccg gtg tac aac cct gag gtg gtg aca gcc tac cga ggc aaa      549
Trp Leu Pro Val Tyr Asn Pro Glu Val Val Thr Ala Tyr Arg Gly Lys
             135             140             145

aag cgc cag gag gcc cca ccc cat atc ttc tcc atc tct gac aat gcc      597
Lys Arg Gln Glu Ala Pro Pro His Ile Phe Ser Ile Ser Asp Asn Ala
         150             155             160

tat cag ttc atg cta act gat cgt gaa aac cag tca atc ttg att act      645
Tyr Gln Phe Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu Ile Thr
         165             170             175

gga gaa tct ggt gca ggg aag act gtg aac acg aag cgt gtc atc cag      693
Gly Glu Ser Gly Ala Gly Lys Thr Val Asn Thr Lys Arg Val Ile Gln
    180             185             190

tac ttt gca aca att gca gtt act gga gag aag aaa aaa gag gaa cct      741
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Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu Glu Pro
195   200   205   210

gcc tct ggc aaa atg cag ggg acc att gaa gat caa atc atc agt gct   789
Ala Ser Gly Lys Met Gln Gly Thr Leu Glu Asp Gln Ile Ile Ser Ala
                215   220   225

aac ccc cta ctg gaa gcc ttc ggc aat gcc aag acc gtg agg aat gac   837
Asn Pro Leu Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg Asn Asp
            230   235   240

aac tcc tct cgc ttt ggt aaa ttc atc agg atc cat ttt ggt gcc aca   885
Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly Ala Thr
        245   250   255

ggc aaa ctg gct tct gca gat att gaa aca tat ctg cta gag aag tcc   933
Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu Lys Ser
    260   265   270

cga gtt act ttt cag cta aag gct gaa aga agc tac cac ata ttt tat   981
Arg Val Thr Phe Gln Leu Lys Ala Glu Arg Ser Tyr His Ile Phe Tyr
275   280   285   290

caa atc ctg tcc aat aag aaa cca gag ctc att gaa atg ctt ctg atc   1029
Gln Ile Leu Ser Asn Lys Lys Pro Glu Leu Ile Glu Met Leu Leu Ile
                295   300   305

acc acc aac cca tat gac ttc gca ttt gtc agc caa ggg gaa att act   1077
Thr Thr Asn Pro Tyr Asp Phe Ala Phe Val Ser Gln Gly Glu Ile Thr
            310   315   320

gtg ccc agc att gat gac cag gaa gag ctg atg gcc aca gat agt gct   1125
Val Pro Ser Ile Asp Asp Gln Glu Glu Leu Met Ala Thr Asp Ser Ala
        325   330   335

gtg gac atc ctg ggt ttc act gct gat gaa aag gtg gcc att tac aag   1173

Val Asp Ile Leu Gly Phe Thr Ala Asp Glu Lys Val Ala Ile Tyr Lys
    340   345   350

ctc act gga gcc gtg atg cat tat ggg aac atg aaa ttc aag caa aag   1221
Leu Thr Gly Ala Val Met His Tyr Gly Asn Met Lys Phe Lys Gln Lys
355   360   365   370

caa agg gaa gag cag gca gag cca gat ggc acg gaa gtt gct gac aaa   1269
Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Val Ala Asp Lys
                375   380   385

gct gct tat ctg aca agt ctg aac tct gct gac ctg ctc aaa tct ctc   1317
Ala Ala Tyr Leu Thr Ser Leu Asn Ser Ala Asp Leu Leu Lys Ser Leu
            390   395   400

tgc tat ccc aga gtc aag gtc ggc aat gag ttc gta acc aaa ggc cag   1365
Cys Tyr Pro Arg Val Lys Val Gly Asn Glu Phe Val Thr Lys Gly Gln
        405   410   415

act gtg cag cag gtg tac aac gca gtg ggt gct ctg gcc aaa gcc atc   1413
Thr Val Gln Gln Val Tyr Asn Ala Val Gly Ala Leu Ala Lys Ala Ile
    420   425   430

tac gag aag atg ttc ctg tgg atg gtc acc cgc atc aac cag cag ctg   1461
Tyr Glu Lys Met Phe Leu Trp Met Val Thr Arg Ile Asn Gln Gln Leu
435   440   445   450

gac acc aag cag ccc agg cag tac ttc atc ggg gtc ttg gac att gct   1509
Asp Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp Ile Ala
                455   460   465

ggc ttt gag atc ttt gat ttc aac agc ctg gag cag ctg tgc atc aac   1557
Gly Phe Glu Ile Phe Asp Phe Asn Ser Leu Glu Gln Leu Cys Ile Asn
            470   475   480

ttc acc aac gag aag ctg caa cag ttt ttc aac cac cac atg ttc gtg   1605

Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met Phe Val
      485   490   495

ctg gag cag gaa gag tac aag aag gaa ggc atc gag tgg gag ttc att   1653
Leu Glu Gln Glu Glu Tyr Lys Lys Glu Gly Ile Glu Trp Glu Phe Ile
500   505   510

gac ttc ggg atg gac ctg gct gcc tgc atc gag ctc atc gag aag cct   1701
Asp Phe Gly Met Asp Leu Ala Ala Cys Ile Glu Leu Ile Glu Lys Pro
515   520   525   530

atg ggc atc ttc tcc atc cta gaa gag gag tgc atg ttc ccc aag gca   1749
Met Gly Ile Phe Ser Ile Leu Glu Glu Glu Cys Met Phe Pro Lys Ala
          535   540   545

aca gac acc tcc ttc aag aac aag ctg tat gaa caa cat ctt gga aag   1797
Thr Asp Thr Ser Phe Lys Asn Lys Leu Tyr Glu Gln His Leu Gly Lys
          550   555   560

tcc aac aac ttc cag aag ccc aag cct gcc aaa ggc aag cct gag gct   1845
Ser Asn Asn Phe Gln Lys Pro Lys Pro Ala Lys Gly Lys Pro Glu Ala
          565   570   575

cac ttc tca ctg gtg cac tat gcc ggc acc gtg gac tac aac atc gcc   1893
His Phe Ser Leu Val His Tyr Ala Gly Thr Val Asp Tyr Asn Ile Ala
580   585   590

ggc tgg ctg gac aaa aac aag gac ccc ctg aat gag act gtg gtg ggg   1941
Gly Trp Leu Asp Lys Asn Lys Asp Pro Leu Asn Glu Thr Val Val Gly
595   600   605   610

ctg tac cag aag tct gca atg aag act ctg gct ttc ctc ttc tct ggg   1989
Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Phe Leu Phe Ser Gly
          615   620   625

gca caa act gct gaa gca gag ggt ggt ggt gga aag aaa ggt ggc aaa   2037

Ala Gln Thr Ala Glu Ala Glu Gly Gly Gly Gly Lys Lys Gly Gly Lys
       630   635   640

aag aag ggt tct tct ttc cag aca gtg tca gct ctt ttc agg gag aat   2085
Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu Phe Arg Glu Asn
       645   650   655

ttg aat aag ctg atg acc aac ttg agg agc act cac cca cac ttt gtg   2133
Leu Asn Lys Leu Met Thr Asn Leu Arg Ser Thr His Pro His Phe Val
       660   665   670

cgg tgc atc atc ccc aat gaa act aaa act cct ggt gcc atg gag cat   2181
Arg Cys Ile Ile Pro Asn Glu Thr Lys Thr Pro Gly Ala Met Glu His
675   680   685   690

gag ctt gtc ctg cat cag ctg agg tgt aac ggt gtg ctg gaa ggc atc   2229
Glu Leu Val Leu His Gln Leu Arg Cys Asn Gly Val Leu Glu Gly Ile
          695   700   705

cgc atc tgc agg aaa ggc ttc cca agc aga atc ctt tat gca gac ttc   2277
Arg Ile Cys Arg Lys Gly Phe Pro Ser Arg Ile Leu Tyr Ala Asp Phe
          710   715   720

aaa cag aga tac aag gtt cta aat gcg agt gct atc cca gag ggt cag   2325
Lys Gln Arg Tyr Lys Val Leu Asn Ala Ser Ala Ile Pro Glu Gly Gln
     725   730   735

ttc att gac agc aag aag gct tct gag aaa ctt cta ggg tct att gaa   2373
Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu Gly Ser Ile Glu
     740   745   750

att gac cac acc cag tac aaa ttc ggt cat acc aag gtt ttc ttc aaa   2421
Ile Asp His Thr Gln Tyr Lys Phe Gly His Thr Lys Val Phe Phe Lys
755   760   765   770

gct ggc ctg ctg gga act cta gaa gaa atg cga gat gaa aag cta gct   2469

Ala Gly Leu Leu Gly Thr Leu Glu Glu Met Arg Asp Glu Lys Leu Ala
                775   780   785

caa ctc atc acg cgc act caa gcc ata tgc agg ggg ttc ctg atg aga   2517
Gln Leu Ile Thr Arg Thr Gln Ala Ile Cys Arg Gly Phe Leu Met Arg
                790   795   800

gtg gag ttc aga aag atg atg gag agg aga gag tcc atc ttc tgc att   2565
Val Glu Phe Arg Lys Met Met Glu Arg Arg Glu Ser Ile Phe Cys Ile
        805   810   815

cag tac aac atc cgt gct ttc atg aat gtg aag cac tgg ccc tgg atg   2613
Gln Tyr Asn Ile Arg Ala Phe Met Asn Val Lys His Trp Pro Trp Met
        820   825   830

aag ctg tat ttc aag atc aag ccc ctc ctc aag agt gca gag aca gag   2661
Lys Leu Tyr Phe Lys Ile Lys Pro Leu Leu Lys Ser Ala Glu Thr Glu
835   840   845   850

aag gag atg goo aac atg aag gag gag ttt gag aaa acc aaa gaa gag   2709
Lys Glu Met Ala Asn Met Lys Glu Glu Phe Glu Lys Thr Lys Glu Glu
                855   860   865

ctg gct aag aca gag gca aaa agg aaa gaa cta gaa gaa aag atg gtg   2757
Leu Ala Lys Thr Glu Ala Lys Arg Lys Glu Leu Glu Glu Lys Met Val
                870   875   880

acg cta atg caa gag aaa aat gac tta caa ctc caa gtt caa gct gaa   2805
Thr Leu Met Gln Glu Lys Asn Asp Leu Gln Leu Gln Val Gln Ala Glu
        885   890   895

gca gat goo ttg gct gat gca gag gaa aga tgt gat cag ttg att aaa   2853
Ala Asp Ala Leu Ala Asp Ala Glu Glu Arg Cys Asp Gln Leu Ile Lys
        900   905   910

acc aaa atc caa ctt gag gcc aaa atc aaa gag gta act gaa aga gct   2901

Thr Lys Ile Gln Leu Glu Ala Lys Ile Lys Glu Val Thr Glu Arg Ala
915   920   925   930

gag gat gag gaa gag atc aat gct gag ctg aca gcc aag aag agg aaa   2949
Glu Asp Glu Glu Glu Ile Asn Ala Glu Leu Thr Ala Lys Lys Arg Lys
                  935   940   945

ctg gag gat gaa tgt tca gag ctc aag aaa gac att gat gac ctt gag   2997
Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile Asp Asp Leu Glu
         950   955   960

ctg aca ctg gcc aag gtt gag aag gag aaa cat gcc acc gag aac aag   3045
Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala Thr Glu Asn Lys
      965   970   975

gtg aaa aac ctc aca gag gag atg gca ggt ctg gat gaa acc att gct   3093
Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp Glu Thr Ile Ala
   980   985   990

aag ctg acc aag gag aag aag gct ctc cag gag gcc cac cag cag   3138
Lys Leu Thr Lys Glu Lys Lys Ala Leu Gln Glu Ala His Gln Gln
995   1000   1005

acc ctg gat gac ctg cag atg gag gag gac aaa gtc aac acc ctg   3183
Thr Leu Asp Asp Leu Gln Met Glu Glu Asp Lys Val Asn Thr Leu
1010   1015   1020

acc aaa gct aaa acc aag cta gaa cag caa gtg gac gat ctt gaa   3228
Thr Lys Ala Lys Thr Lys Leu Glu Gln Gln Val Asp Asp Leu Glu
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gga tct ctg gaa caa gaa aag aaa ctt tgc atg gac tta gaa aga   3273
Gly Ser Leu Glu Gln Glu Lys Lys Leu Cys Met Asp Leu Glu Arg
1040   1045   1050

gcc aag aga aaa ctg gag ggt gac cta aaa ttg gcc caa gaa tcc   3318

Ala Lys Arg Lys Leu Glu   Gly Asp Leu Lys Leu   Ala Gln Glu Ser
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aca   atg gat aca gaa aat   gac aaa cag caa ctt   aat gag aaa ctc   3363
Thr   Met Asp Thr Glu Asn   Asp Lys Gln Gln Leu   Asn Glu Lys Leu
1070   1075   1080

aaa   aag aaa gag ttt gaa   atg agc aat ctg caa   ggc aag att gaa   3408
Lys   Lys Lys Glu Phe Glu   Met Ser Asn Leu Gln   Gly Lys Ile Glu
1085   1090   1095

gat   gaa caa gcc ctt gca   atg cag cta caa aag   aag atc aaa gaa   3453
Asp   Glu Gln Ala Leu Ala   Met Gln Leu Gln Lys   Lys Ile Lys Glu
1100   1105   1110

tta   cag gcc cgc att gag   gag ctg gag gag gaa   atc gag gca gag   3498
Leu   Gln Ala Arg Ile Glu   Glu Leu Glu Glu Glu   Ile Glu Ala Glu
1115   1120   1125

cgg   gcc tcc cgg gcc aaa   gca gaa aag cag cgc   tct gac ctc tcc   3543
Arg   Ala Ser Arg Ala Lys   Ala Glu Lys Gln Arg   Ser Asp Leu Ser
1130   1135   1140

cgg   gag ctg gag gag atc   agt gag agg ctg gaa   gaa gcc ggt gag   3588
Arg   Glu Leu Glu Glu Ile   Ser Glu Arg Leu Glu   Glu Ala Gly Gly
1145   1150   1155

gcc   act tca gcc cag att   gag ttg aac aag aag   cgg gag gct gag   3633
Ala   Thr Ser Ala Gln Ile   Glu Leu Asn Lys Lys   Arg Glu Ala Glu
1160   1165   1170

ttc   cag aaa atg cgc agg   gac ctg gaa gag tcc   acc ctg cag cac   3678
Phe   Gln Lys Met Arg Arg   Asp Leu Glu Glu Ser   Thr Leu Gln His
1175   1180   1185

gaa   gcc aag gca gct gct   ctt cgg aag aag cac   gca gat agt gtg   3723

Glu Ala Thr Ala Ala Ala Leu Arg Lys Lys His Ala Asp Ser Val
1190   1195   1200

gct gag ctt gga aag cag atc gac agc ctt cag cgg gtc aag cag   3768
Ala Glu Leu Gly Lys Gln Ile Asp Ser Leu Gln Arg Val Lys Gln
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Lys Leu Glu Lys Glu Lys Ser Glu Leu Lys Met Glu Ile Asn Asp
1220   1225   1230

ctt gct agt aac atg gag act gtc tcc aaa gcc aag gca aac ttt   3858
Leu Ala Ser Asn Met Glu Thr Val Ser Lys Ala Lys Ala Asn Phe
1235   1240   1245

gag aaa atg tgc cgc acc cta gag gac cag ctt agt gaa ata aaa   3903
Glu Lys Met Cys Arg Thr Leu Glu Asp Gln Leu Ser Glu Ile Lys
1250   1255   1260

aca aag gaa gag gag cag caa cgc tta ata aat gag ttg tca gcc   3948
Thr Lys Glu Glu Glu Gln Gln Arg Leu Ile Asn Glu Leu Ser Ala
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cag aag gca cgt tta cac aca gaa tca ggt gag ttt tca cga cag   3993
Gln Lys Ala Arg Leu His Thr Glu Ser Gly Glu Phe Ser Arg Gln
1280   1285   1290

cta gat gaa aaa gat gct atg gtt tct cag ctc tcc cga ggc aaa   4038
Leu Asp Glu Lys Asp Ala Met Val Ser Gln Leu Ser Arg Gly Lys
1295   1300   1305

caa gca ttt aca caa cag att gaa gaa tta aag agg cag cta gaa   4083
Gln Ala Phe Thr Gln Gln Ile Glu Glu Leu Lys Arg Gln Leu Glu
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gag gag act aag gcc aag agc act ctg gcc cat gcc ctg cag tca   4128

Glu Glu Thr Lys Ala Lys Ser Thr Leu Ala His Ala Leu Gln Ser
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Ala Arg His Asp Cys Asp Leu Leu Arg Glu Gln Tyr Glu Glu Glu
1340 1345 1350

cag gaa gcc aag gct gag ctg cag agg gaa atg tcc aag gcc aac   4218
Gln Glu Ala Lys Ala Glu Leu Gln Arg Glu Met Ser Lys Ala Asn
1355 1360 1365

agt gag gtt gcc cag tgg agg acc aag tac gag acg gac gcc atc   4263
Ser Glu Val Ala Gln Trp Arg Thr Lys Tyr Glu Thr Asp Ala Ile
1370 1375 1380

cag cgc aca gag gag ctg gag gag gcc aag aag aag cta gcc cag   4308
Gln Arg Thr Glu Glu Leu Glu Glu Ala Lys Lys Lys Leu Ala Gln
1385 1390 1395

cgt ctg cag gat gca gaa gaa cat gta gaa gct gtg aat tcc aaa   4353
Arg Leu Gln Asp Ala Glu Glu His Val Glu Ala Val Asn Ser Lys
1400 1405 1410

tgt gct tct ctt gaa aag aca aag cag agg cta cag aat gaa gta   4398
Cys Ala Ser Leu Glu Lys Thr Lys Gln Arg Leu Gln Asn Glu Val
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gag gac ctc atg att gat gtg gaa cga tct aat gct gcc tgc ata   4443
Glu Asp Leu Met Ile Asp Val Glu Arg Ser Asn Ala Ala Cys Ile
1430 1435 1440

gct ctc gat aag aag caa aga aac ttt gac aag gtt ctg gca gaa   4488
Ala Leu Asp Lys Lys Gln Arg Asn Phe Asp Lys Val Leu Ala Glu
1445 1450 1455

tgg aaa cag aag tat gag gaa act cag gct gaa ctt gag gcc tcc   4533

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Trp  Lys Gln Lys Tyr Glu  Glu Thr Gln Ala Glu  Leu Glu Ala Ser
1460                 1465                 1470

cag  aag gag tcg cgt tct  ctc agc act gag ctg  ttc aag gtg aag       4578
Gln  Lys Glu Ser Arg Ser  Leu Ser Thr Glu Leu  Phe Lys Val Lys
1475                 1480                 1485

aat  gcc tac gag gaa tcc  ctg gat cat ctt gaa  act cta aag cga       4623
Asn  Ala Tyr Glu Glu Ser  Leu Asp His Leu Glu  Thr Leu Lys Arg
1490                 1495                 1500

gag  aat aag aac tta caa  cag gag att tct gac  ctg aca gag caa       4668
Glu  Asn Lys Asn Leu Gln  Gln Glu Ile Ser Asp  Leu Thr Glu Gln
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att  gca gag ggt gga aag  cat atc cat gaa ctg  gag aaa gta aag  ·   4713
Ile  Ala Glu Gly Gly Lys  His Ile His Glu Leu  Glu Lys Val Lys
1520                 1525                 1530

aaa  caa ctt gat cat gag  aag agt gaa cta cag  act tcc cta gag       4758
Lys  Gln Leu Asp His Glu  Lys Ser Glu Leu Gln  Thr Ser Leu Glu
1535                 1540                 1545

gaa  gca gag gca tct ctt  gag cat gaa gaa ggc  aaa att ctt cgc       4803
Glu  Ala Glu Ala Ser Leu  Glu His Glu Glu Gly  Lys Ile Leu Arg
1550                 1555                 1560

att  caa ctt gag cta aat  cag gtg aaa tct gag  att gac cga aaa       4848
Ile  Gln Leu Glu Leu Asn  Gln Val Lys Ser Glu  Ile Asp Arg Lys
1565                 1570                 1575

att  gct gaa aaa gat gaa  gaa ctc gat cag cta  aag agg aac cat       4893
Ile  Ala Glu Lys Asp Glu  Glu Leu Asp Gln Leu  Lys Arg Asn His
1580                 1585                 1590

ctc  aga gtt gtg gag tca  atg cag agt aca ctg  gat gct gag atc       4938
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Leu Arg Val Val Glu Ser Met Gln Ser Thr Leu Asp Ala Glu Ile
1595   1600   1605

agg agc aga aat gat gct ctg agg atc aag aag aag atg gag gga   4983
Arg Ser Arg Asn Asp Ala Leu Arg Ile Lys Lys Lys Met Glu Gly
1610   1615   1620

gat ctt aat gaa atg gaa atc cag ctg aac cat gcc aac cgc cag   5028
Asp Leu Asn Glu Met Glu Ile Gln Leu Asn His Ala Asn Arg Gln
1625   1630   1635

gct gct gag gca cta agg aat ctt aag aac aca caa gga ata ctg   5073
Ala Ala Glu Ala Leu Arg Asn Leu Arg Asn Thr Gln Gly Ile Leu
1640   1645   1650

aag gac act cag cta cat ttg gat gat gcc atc aga ggc caa gat   5118
Lys Asp Thr Gln Leu His Leu Asp Asp Ala Ile Arg Gly Gln Asp
1655   1660   1665

gac ctt aag gaa caa ttg gca atg gtt gag cgc aga gct aac ctg   5163
Asp Leu Lys Glu Gln Leu Ala Met Val Glu Arg Arg Ala Asn Leu
1670   1675   1680

atg cag gct gaa gtt gaa gag ctc agg gca tcc ctg gaa cgg act   5208
Met Gln Ala Glu Val Glu Glu Leu Arg Ala Ser Leu Glu Arg Thr
1685   1690   1695

gag aga ggc agg aaa atg gca gag caa gag ctt ctg gat gcc agt   5253
Glu Arg Gly Arg Lys Met Ala Glu Gln Glu Leu Leu Asp Ala Ser
1700   1705   1710

gaa cgt gtg caa ctt ctg cac act cag aac aca agc ctg atc aac   5298
Glu Arg Val Gln Leu Leu His Thr Gln Asn Thr Ser Leu Ile Asn
1715   1720   1725

acc aag aag aag ctg gaa aca gac att tcc caa atc cag gga gag   5343

Thr Lys Lys Lys Leu Glu Thr Asp Ile Ser Gln Ile Gln Gly Glu
1730 1735 1740

atg gag gac atc gtc cag gaa gcc cgc aat gca gag gag aag gcc 5388
Met Glu Asp Ile Val Gln Glu Ala Arg Asn Ala Glu Glu Lys Ala
1745 1750 1755

aag aag gcc atc act gat gct gcc atg atg gct gag gag ctg aag 5433
Lys Lys Ala Ile Thr Asp Ala Ala Met Met Ala Glu Glu Leu Lys
1760 1765 1770

aag gaa cag gac acc agc gcc cac ctg gag cgg atg aag aag aac 5478
Lys Glu Gln Asp Thr Ser Ala His Leu Glu Arg Met Lys Lys Asn
1775 1780 1785

atg gag cag acc gtg aag gat ctg cag ctc cgt ctg ggt gag gct 5523
Met Glu Gln Thr Val Lys Asp Leu Gln Leu Arg Leu Gly Glu Ala
1790 1795 1800

gag cag ctg gcg ctg aag ggt ggg aag aag cag atc cag aaa ctg 5568
Glu Gln Leu Ala Leu Lys Gly Gly Lys Lys Gln Ile Gln Lys Leu
1805 1810 1815

gag gcc agg gtg aga gag ctt gaa agt gag gtg gaa agt gaa cag 5613
Glu Ala Arg Val Arg Glu Leu Glu Ser Glu Val Glu Ser Glu Gln
1820 1825 1830

aag cac aat gtt gag gct gtc aag ggt ctt cgc aaa cat gag aga 5658
Lys His Asn Val Glu Ala Val Lys Gly Leu Arg Lys His Glu Arg
1835 1840 1845

aga gtg aag gaa ctc act tac cag act gag gag gac cgc aag aat 5703
Arg Val Lys Glu Leu Thr Tyr Gln Thr Glu Glu Asp Arg Lys Asn
1850 1855 1860

att ctc agg ctg cag gac ttg gtg gac aaa ttg caa acc aaa gtc 5748

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Ile  Leu Arg Leu Gln Asp  Leu Val Asp Lys Leu  Gln Thr Lys Val
1865                 1870               1875


aaa  gct tac aag aga caa  gct gaa gag gct gag  gaa caa tcc aat   5793
Lys  Ala Tyr Lys Arg Gln  Ala Glu Glu Ala Glu  Glu Gln Ser Asn
1880                 1885               1890


gtc  aac ctt gcc aag ttc  cgc aag ctc cag cac  gag ctg gag gag   5838
Val  Asn Leu Ala Lys Phe  Arg Lys Leu Gln His  Glu Leu Glu Glu
1895                 1900               1905


gcc  gag gaa ggg gct gac  att gct gag tcc caa  gtc aac aag ctg   5883
Ala  Glu Glu Arg Ala Asp  Ile Ala Glu Ser Gln  Val Asn Lys Leu
1910                 1915               1920


aga  gtg aag agt ggg gag  gtt cac aca uaa gtc  ata agt gaa gag , -5928
Arg  Val Lys Ser Arg Glu  Val His Thr Lys Val  Ile Ser Glu Glu
1925                 1930               1935


taa ttcattctaa tgaaagaaaa tgtgaccaas gaaatgcacg aaatgtgaag        5981


ttctttgtca ctgtcctgta tatcaaggaa ataaa                           6016



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<212>  PRT
<213>  Homo sapiens


<400>  4


Met Ser Ser Asp Ser Glu Met Ala Ile Phe Gly Glu Ala Ala Pro Phe
1               5                 10                15



Leu Arg Lys Ser Glu Lys Glu Arg Ile Glu Ala Gln Asn Lys Pro Phe
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　　　　　　　20　　　　　　　　　25　　　　　　　　30

Asp Ala Lys Thr Ser Val Phe Val Val Asp Pro Lys Glu Ser Tyr Val
　　　　35　　　　　　　　　　40　　　　　　　　　45

Lys Ala Ile Val Gln Ser Arg Glu Gly Gly Lys Val Thr Ala Lys Thr
　　50　　　　　　　　　55　　　　　　　　60

Glu Ala Gly Ala Thr Val Thr Val Lys Glu Asp Gln Val Phe Ser Met
65　　　　　　　　　70　　　　　　　　75　　　　　　　80

Asn Pro Pro Lys Tyr Asp Lys Ile Glu Asp Met Ala Met Met Thr His
　　　　　　　　85　　　　　　　　　90　　　　　　　　95

Leu His Glu Pro Ala Val Leu Tyr Asn Leu Lys Glu Arg Tyr Ala Ala
　　　　　100　　　　　　　　　105　　　　　　　　110

Trp Met Ile Tyr Thr Tyr Ser Gly Leu Phe Cys Val Thr Val Asn Pro
　　　　115　　　　　　　　　120　　　　　　　　125

Tyr Lys Trp Leu Pro Val Tyr Asn Pro Glu Val Val Thr Ala Tyr Arg
　　130　　　　　　　　135　　　　　　　　140

Gly Lys Lys Arg Gln Glu Ala Pro Pro His Ile Phe Ser Ile Ser Asp
145　　　　　　　　150　　　　　　　　155　　　　　　　160

Asn Ala Tyr Gln Phe Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu

165   170   175

Ile Thr Gly Glu Ser Gly Ala Gly Lys Thr Val Asn Thr Lys Arg Val
         180   185   190

Ile Gln Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu
         195   200   205

Glu Pro Ala Ser Gly Lys Met Gln Gly Thr Leu Glu Asp Gln Ile Ile
     210   215   220

Ser Ala Asn Pro Leu Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg
225   230   235   240

Asn Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly
             245   250   255

Ala Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu
             260   265   270

Lys Ser Arg Val Thr Phe Gln Leu Lys Ala Glu Arg Ser Tyr His Ile
             275   280   285

Phe Tyr Gln Ile Leu Ser Asn Lys Lys Pro Glu Leu Ile Glu Met Leu
     290   295   300

Leu Ile Thr Thr Asn Pro Tyr Asp Phe Ala Phe Val Ser Gln Gly Glu

305 310 315 320

Ile Thr Val Pro Ser Ile Asp Asp Gln Glu Glu Leu Met Ala Thr Asp
325 330 335

Ser Ala Val Asp Ile Leu Gly Phe Thr Ala Asp Glu Lys Val Ala Ile
340 345 350

Tyr Lys Leu Thr Gly Ala Val Met His Tyr Gly Asn Met Lys Phe Lys
355 360 365

Gln Lys Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Val Ala
370 375 380

Asp Lys Ala Ala Tyr Leu Thr Ser Leu Asn Ser Ala Asp Leu Leu Lys
385 390 395 400

Ser Leu Cys Tyr Pro Arg Val Lys Val Gly Asn Glu Phe Val Thr Lys
405 410 415

Gly Gln Thr Val Gln Gln Val Tyr Asn Ala Val Gly Ala Leu Ala Lys
420 425 430

Ala Ile Tyr Glu Lys Met Phe Leu Trp Met Val Thr Arg Ile Asn Gln
435 440 445

Gln Leu Asp Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp

              450   455   460

Ile Ala Gly Phe Glu Ile Phe Asp Phe Asn Ser Leu Glu Gln Leu Cys
465   470   475   480

Ile Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met
                485   490   495

Phe Val Leu Glu Gln Glu Glu Tyr Lys Lys Glu Gly Ile Glu Trp Glu
                500   505   510

Phe Ile Asp Phe Gly Met Asp Leu Ala Ala Cys Ile Glu Leu Ile Glu
        515   520   525

Lys Pro Met Gly Ile Phe Ser Ile Leu Glu Glu Glu Cys Met Phe Pro
    530   535   540

Lys Ala Thr Asp Thr Ser Phe Lys Asn Lys Leu Tyr Glu Gln His Leu
545   550   555   560

Gly Lys Ser Asn Asn Phe Gln Lys Pro Lys Pro Ala Lys Gly Lys Pro
                565   570   575

Glu Ala His Phe Ser Leu Val His Tyr Ala Gly Thr Val Asp Tyr Asn
                580   585   590

Ile Ala Gly Trp Leu Asp Lys Asn Lys Asp Pro Leu Asn Glu Thr Val

                595   600   605

Val Gly Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Phe Leu Phe
    610   615   620

Ser Gly Ala Gln Thr Ala Glu Ala Glu Gly Gly Gly Gly Lys Lys Gly
625   630   635   640

Gly Lys Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu Phe Arg
                645   650   655

Glu Asn Leu Asn Lys Leu Met Thr Asn Leu Arg Ser Thr His Pro His
                660   665   670

Phe Val Arg Cys Ile Ile Pro Asn Glu Thr Lys Thr Pro Gly Ala Met
        675   680   685

Glu His Glu Leu Val Leu His Gln Leu Arg Cys Asn Gly Val Leu Glu
    690   695   700

Gly Ile Arg Ile Cys Arg Lys Gly Phe Pro Ser Arg Ile Leu Tyr Ala
705   710   715   720

Asp Phe Lys Gln Arg Tyr Lys Val Leu Asn Ala Ser Ala Ile Pro Glu
                725   730   735

Gly Gln Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu Gly Ser

                    740   745   750

Ile Glu Ile Asp His Thr Gln Tyr Lys Phe Gly His Thr Lys Val Phe
                    755   760   765

Phe Lys Ala Gly Leu Leu Gly Thr Leu Glu Glu Met Arg Asp Glu Lys
          770   775   780

Leu Ala Gln Leu Ile Thr Arg Thr Gln Ala Ile Cys Arg Gly Phe Leu
785   790   795   800

Met Arg Val Glu Phe Arg Lys Met Met Glu Arg Arg Glu Ser Ile Phe
                    805   810   815

Cys Ile Gln Tyr Asn Ile Arg Ala Phe Met Asn Val Lys His Trp Pro
          820   825   830

Trp Met Lys Leu Tyr Phe Lys Ile Lys Pro Leu Leu Lys Ser Ala Glu
          835   840   845

Thr Glu Lys Glu Met Ala Asn Met Lys Glu Glu Phe Glu Lys Thr Lys
          850   855   860

Glu Glu Leu Ala Lys Thr Glu Ala Lys Arg Lys Glu Leu Glu Glu Lys
865   870   875   880

Met Val Thr Leu Met Gln Glu Lys Asn Asp Leu Gln Leu Gln Val Gln

                    885   890   895

Ala Glu Ala Asp Ala Leu Ala Asp Ala Glu Glu Arg Cys Asp Gln Leu
            900   905   910

Ile Lys Thr Lys Ile Gln Leu Glu Ala Lys Ile Lys Glu Val Thr Glu
        915   920   925

Arg Ala Glu Asp Glu Glu Glu Ile Asn Ala Glu Leu Thr Ala Lys Lys
    930   935   940

Arg Lys Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile Asp Asp
945   950   955   960

Leu Glu Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala Thr Glu
            965   970   975

Asn Lys Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp Glu Thr
        980   985   990

Ile Ala Lys Leu Thr Lys Glu Lys Lys Ala Leu Gln Glu Ala His Gln
        995   1000   1005

Gln Thr Leu Asp Asp Leu Gln Met Glu Glu Asp Lys Val Asn Thr
    1010   1015   1020

Leu Thr Lys Ala Lys Thr Lys Leu Glu Gln Gln Val Asp Asp Leu

1025   1030   1035

Glu Gly Ser Leu Glu Gln Glu Lys Lys Leu Cys Met Asp Leu Glu
    1040   1045   1050

Arg Ala Lys Arg Lys Leu Glu Gly Asp Leu Lys Leu Ala Gln Glu
    1055   1060   1065

Ser Thr Met Asp Thr Glu Asn Asp Lys Gln Gln Leu Asn Glu Lys
    1070   1075   1080

Leu Lys Lys Lys Glu Phe Glu Met Ser Asn Leu Gln Gly Lys Ile
    1085   1090   1095

Glu Asp Glu Gln Ala Leu Ala Met Gln Leu Gln Lys Lys Ile Lys
    1100   1105   1110

Glu Leu Gln Ala Arg Ile Glu Glu Leu Glu Glu Glu Ile Glu Ala
    1115   1120   1125

Glu Arg Ala Ser Arg Ala Lys Ala Glu Lys Gln Arg Ser Asp Leu
    1130   1135   1140

Ser Arg Glu Leu Glu Glu Ile Ser Glu Arg Leu Glu Glu Ala Gly
    1145   1150   1155

Gly Ala Thr Ser Ala Gln Ile Glu Leu Asn Lys Lys Arg Glu Ala

1160   1165   1170

Glu Phe Gln Lys Met Arg Arg Asp Leu Glu Glu Ser Thr Leu Gln
    1175   1180   1185

His Glu Ala Thr Ala Ala Ala Leu Arg Lys Lys His Ala Asp Ser
    1190   1195   1200

Val Ala Glu Leu Gly Lys Gln Ile Asp Ser Leu Gln Arg Val Lys
    1205   1210   1215

Gln Lys Leu Glu Lys Glu Lys Ser Glu Leu Lys Met Glu Ile Asn
    1220   1225   1230

Asp Leu Ala Ser Asn Met Glu Thr Val Ser Lys Ala Lys Ala Asn
    1235   1240   1245

Phe Glu Lys Met Cys Arg Thr Leu Glu Asp Gln Leu Ser Glu Ile
    1250   1255   1260

Lys Thr Lys Glu Glu Glu Gln Gln Arg Leu Ile Asn Glu Leu Ser
    1265   1270   1275

Ala Gln Lys Ala Arg Leu His Thr Glu Ser Gly Glu Phe Ser Arg
    1280   1285   1290

Gln Leu Asp Glu Lys Asp Ala Met Val Ser Gln Leu Ser Arg Gly

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        1295              1300              1305

Lys Gln  Ala Phe Thr Gln Gln  Ile Glu Glu Leu Lys  Arg Gln Leu
     1310             1315             1320


Glu Glu  Glu Thr Lys Ala Lys  Ser Thr Leu Ala His  Ala Leu Gln
     1325             1330             1335


Ser Ala  Arg His Asp Cys Asp  Leu Leu Arg Glu Gln  Tyr Glu Glu
     1340             1345             1350


Glu Gln  Glu Ala Lys Ala Glu  Leu Gln Arg Gly Met  Ser Lys Ala
     1355             1360             1365


Asn Ser  Glu Val Ala Gln Trp  Arg Thr Lys Tyr Glu  Thr Asp Ala
     1370             1375             1380


Ile Gln  Arg Thr Glu Glu Leu  Glu Glu Ala Lys Lys  Lys Leu Ala
     1385             1390             1395


Gln Arg  Leu Gln Asp Ala Glu  Glu His Val Glu Ala  Val Asn Ser
     1400             1405             1410


Lys Cys  Ala Ser Leu Glu Lys  Thr Lys Gln Arg Leu  Gln Asn Glu
     1415             1420             1425


Val Glu  Asp Leu Met Ile Asp  Val Glu Arg Ser Asn  Ala Ala Cys
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                1430   1435   1460

    Ile Ala Leu Asp Lys Lys Gln Arg Asn Phe Asp Lys Val Leu Ala
          1445   1450   1455

    Glu Trp Lys Gln Lys Tyr Glu Glu Thr Gln Ala Glu Leu Glu Ala
          1460   1465   1470

    Ser Gln Lys Glu Ser Arg Ser Leu Ser Thr Glu Leu Phe Lys Val
          1475   1480   1485

    Lys Asn Ala Tyr Glu Glu Ser Leu Asp His Leu Glu Thr Leu Lys
          1490   1495   1500

    Arg Glu Asn Lys Asn Leu Gln Gln Glu Ile Ser Asp Leu Thr Glu
          1505   1510   1515

    Gln Ile Ala Glu Gly Gly Lys His Ile His Glu Leu Glu Lys Val
          1520   1525   1530

    Lys Lys Gln Leu Asp His Glu Lys Ser Glu Leu Gln Thr Ser Leu
          1535   1540   1545

    Glu Glu Ala Glu Ala Ser Leu Glu His Glu Glu Gly Lys Ile Leu
          1550   1555   1560

    Arg Ile Gln Leu Glu Leu Asn Gln Val Lys Ser Glu Ile Asp Arg

    1565   1570   1575

Lys Ile Ala Glu Lys Asp Glu Glu Leu Asp Gln Leu Lys Arg Asn
   1580   1585   1590

His Leu Arg Val Val Glu Ser Met Gln Ser Thr Leu Asp Ala Glu
   1595   1600   1605

Ile Arg Ser Arg Asn Asp Ala Leu Arg Ile Lys Lys Lys Met Glu
   1610   1615   1620

Gly Asp Leu Asn Glu Met Glu Ile Gln Leu Asn His Ala Asn Arg
   1625   1630   1635

Gln Ala Ala Glu Ala Leu Arg Asn Leu Arg Asn Thr Gln Gly Ile
   1640   1645   1650

Leu Lys Asp Thr Gln Leu His Leu Asp Asp Ala Ile Arg Gly Gln
   1655   1660   1665

Asp Asp Leu Lys Glu Gln Leu Ala Met Val Glu Arg Arg Ala Asn
   1670   1675   1680

Leu Met Gln Ala Glu Val Glu Glu Leu Arg Ala Ser Leu Glu Arg
   1685   1690   1695

Thr Glu Arg Gly Arg Lys Met Ala Glu Gln Glu Leu Leu Asp Ala

```
         1700                1705                1710


Ser Glu  Arg Val Gln Leu Leu  His Thr Gln Asn Thr  Ser Leu Ile
    1715                1720                1725


Asn Thr  Lys Lys Lys Leu Glu  Thr Asp Ile Ser Gln  Ile Gln Gly
    1730                1735                1740


Glu Met  Glu Asp Ile Val Gln  Glu Ala Arg Asn Ala  Glu Glu Lys
    1745                1750                1755


Ala Lys  Lys Ala Ile Thr Asp  Ala Ala Met Met Ala  Glu Glu Leu
    1760                1765                1770


Lys Lys  Glu Gln Asp Thr Ser  Ala His Leu Glu Arg  Met Lys Lys
    1775                1780                1785


Asn Met  Glu Gln Thr Val Lys  Asp Leu Gln Leu Arg  Leu Gly Glu
    1790                1795                1800


Ala Glu  Gln Leu Ala Leu Lys  Gly Gly Lys Lys Gln  Ile Gln Lys
    1805                1810                1815


Leu Glu  Ala Arg Val Arg Glu  Leu Glu Ser Glu Val  Glu Ser Glu
    1820                1825                1830


Gln Lys  His Asn Val Glu Ala  Val Lys Gly Leu Arg  Lys His Glu
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1835   1840   1845

Arg Arg Val Lys Glu Leu Thr Tyr Gln Thr Glu Glu Asp Arg Lys
    1850   1855   1860

Asn Ile Leu Arg Leu Gln Asp Leu Val Asp Lys Leu Gln Thr Lys
    1865   1870   1875

Val Lys Ala Tyr Lys Arg Gln Ala Glu Glu Ala Glu Glu Gln Ser
    1880   1885   1890

Asn Val Asn Leu Ala Lys Phe Arg Lys Leu Gln His Glu Leu Glu
    1895   1900   1905

Glu Ala Glu Glu Arg Ala Asp Ile Ala Glu Ser Gln Val Asn Lys
    1910   1915   1920

Leu Arg Val Lys Ser Arg Glu Val His Thr Lys Val Ile Ser Glu
    1925   1930   1935

Glu

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Met Ser Ser Asp Ser Glu Met Ala Ile Phe Gly Glu Ala Ala Pro Phe
1               5                   10                  15

ctc cga aag tct gaa agg gag cga att gaa gcc cag aac aag cct ttt        96
Leu Arg Lys Ser Glu Arg Glu Arg Ile Glu Ala Gln Asn Lys Pro Phe
            20                  25                  30

gat gcc aag aca tca gtc ttt gtg gtg gac cct aag gag tcc ttt gtg       144
Asp Ala Lys Thr Ser Val Phe Val Val Asp Pro Lys Glu Ser Phe Val
        35                  40                  45

aaa gca aca gtg cag agc agg gaa agg ggg aag gtg aca gct aag acc       192
Lys Ala Thr Val Gln Ser Arg Glu Gly Gly Lys Val Thr Ala Lys Thr
    50                  55                  60

gaa gct gga gct act gta aca gtg aaa gat gac caa gtc ttc ccc atg       240
Glu Ala Gly Ala Thr Val Thr Val Lys Asp Asp Gln Val Phe Pro Met
65                  70                  75                  80

aac cct ccc aaa tat gac aag atc gag gac atg gcc atg atg act cat       288
Asn Pro Pro Lys Tyr Asp Lys Ile Glu Asp Met Ala Met Met Thr His
                85                  90                  95

ctc cac gag cct gct gtg ctg tac aac ctc aaa gag cgc tac gca gcc       336
Leu His Glu Pro Ala Val Leu Tyr Asn Leu Lys Glu Arg Tyr Ala Ala
            100                 105                 110

tgg atg atc tac acc tac tca ggc ttg ttc tgt gtc act gtc aac ccc       384
Trp Met Ile Tyr Thr Tyr Ser Gly Leu Phe Cys Val Thr Val Asn Pro
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              115                 120                 125

tac aag tgg ttg cca gtg tat aat gca gaa gtg gtg aca gcc tac cga     432
Tyr Lys Trp Leu Pro Val Tyr Asn Ala Glu Val Val Thr Ala Tyr Arg
    130                 135                 140

ggc aaa aag cgc cag gaa gcc cca cac atc ttc tcc atc tct gac        480
Gly Lys Lys Arg Gln Glu Ala Pro Pro His Ile Phe Ser Ile Ser Asp
145                 150                 155                 160

aat gcc tat cag ttc atg ctg act gat cgg gag aat cag tct atc ttg    528
Asn Ala Tyr Gln Phe Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu
                165                 170                 175

atc acc gga gaa tct ggc gca ggg aag act gtg aac acc aag cgt gtc    576
Ile Thr Gly Glu Ser Gly Ala Gly Lys Thr Val Asn Thr Lys Arg Val
            180                 185                 190

atc cag tac ttt gca aca att gca gtt act ggg gag aag aag aag gaa    624
Ile Gln Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu
            195                 200                 205

gaa gtt act tct ggc aaa atg cag ggg act ctg gaa gat caa atc atc    672
Glu Val Thr Ser Gly Lys Met Gln Gly Thr Leu Glu Asp Gln Ile Ile
    210                 215                 220

agt gcc aac ccc cta ctg gag gcc ttt ggc aac gcc aag acc gtg agg    720
Ser Ala Asn Pro Leu Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg
225                 230                 235                 240

aat gac aac tcc tct cgc ttt ggt aaa ttc atc agg atc cac ttc ggt    768
Asn Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly
                245                 250                 255

acc aca ggg aaa ctg gct tct gct gat att gaa aca tat ctt ctg gag    816
Thr Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu
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                    260   265   270

aag tct aga gtt act ttc cag cta aag gct gaa agc agc tat cat att   864
Lys Ser Arg Val Thr Phe Gln Leu Lys Ala Glu Arg Ser Tyr His Ile
        275   280   285

ttt tat cag atc atg tct aac aag aag cca gat cta att gaa atg ctc   912
Phe Tyr Gln Ile Met Ser Asn Lys Lys Pro Asp Leu Ile Glu Met Leu
    290   295   300

ctg atc acc acc aac cca tac gat tat gcc ttc gtc agt caa ggg gag   960
Leu Ile Thr Thr Asn Pro Tyr Asp Tyr Ala Phe Val Ser Gln Gly Glu
305   310   315   320

atc aca gtg ccc agc att gat gac caa gaa gag ttg atg gct aca gat , ,   1008
Ile Thr Val Pro Ser Ile Asp Asp Gln Glu Glu Leu Met Ala Thr Asp
                325   330   335

agt gcc att gaa att ctg ggc ttt act tca gat gaa aga gtg tcc atc   1056
Ser Ala Ile Glu Ile Leu Gly Phe Thr Ser Asp Glu Arg Val Ser Ile
            340   345   350

tat aag ctc aca ggg gct gtg atg cat tat ggg aac atg aaa ttc aag   1104
Tyr Lys Leu Thr Gly Ala Val Met His Tyr Gly Asn Met Lys Phe Lys
        355   360   365

caa aag cag cgt gag gag caa gct gag cca gat ggc act gaa gtt gct   1152
Gln Lys Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Val Ala
    370   375   380

gac aag gca gcc tat ctc caa aat ctg aac tct gca gat ctg ctc aaa   1200
Asp Lys Ala Ala Tyr Leu Gln Asn Leu Asn Ser Ala Asp Leu Leu Lys
385   390   395   400

gcc ctc tgc tac cct agg gtc aag gtc ggc aat gag tat gtc acc aaa   1248
Ala Leu Cys Tyr Pro Arg Val Lys Val Gly Asn Glu Tyr Val Thr Lys

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                    405              410              415

ggt caa act gtg cag cag gtg tac aat gca gtg ggt gct ctg gcc aaa    1296
Gly Gln Thr Val Gln Gln Val Tyr Asn Ala Val Gly Ala Leu Ala Lys
            420              425              430

gct gtc tac gat aag atg ttc ttg tgg atg gtc acc cgc atc aac cag    1344
Ala Val Tyr Asp Lys Met Phe Leu Trp Met Val Thr Arg Ile Asn Gln
        435              440              445

cag ctg gac acc aag cag ccc agg cag tac ttc att ggg gtc ttg gac    1392
Gln Leu Asp Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp
    450              455              460

att gct ggg ttt gag atc ttt gat ttc aac agc ctg gag cag ctg tgc    1440
Ile Ala Gly Phe Glu Ile Phe Asp Phe Asn Ser Leu Glu Gln Leu Cys
465              470              475              480

atc aac ttc acc aat gag aaa ctg caa cag ttt ttc aac cac cac atg    1488
Ile Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met
            485              490              495

ttc gtg ctg gag cag gag gag tac aag aag gaa ggc att gag tgg acg    1536
Phe Val Leu Glu Gln Glu Glu Tyr Lys Lys Glu Gly Ile Glu Trp Thr
        500              505              510

ttc att gac ttt ggg atg gac ctg gct gcc tgc atc gag ctc atc gag    1584
Phe Ile Asp Phe Gly Met Asp Leu Ala Ala Cys Ile Glu Leu Ile Glu
        515              520              525

aag cct atg ggc atc ttc tcc atc ctg gaa gag gag tgc atg ttc ccc    1632
Lys Pro Met Gly Ile Phe Ser Ile Leu Glu Glu Glu Cys Met Phe Pro
    530              535              540

aag gcg aca gac acc tcc ttc aag aac aag ctg tat gaa caa cat ctt    1680
Lys Ala Thr Asp Thr Ser Phe Lys Asn Lys Leu Tyr Glu Gln His Leu
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      545              550              555              560

gga aaa tcc aat aac ttc cag aag ccc aag cct gcc aaa ggc aag cct   1728
Gly Lys Ser Asn Asn Phe Gln Lys Pro Lys Pro Ala Lys Gly Lys Pro
                565              570              575

aag gcc cac ttc tct ttg att cac tat gct ggc acc gtg gac tac aac   1776
Glu Ala His Phe Ser Leu Ile His Tyr Ala Gly Thr Val Asp Tyr Asn
                590              585              590

att gcc ggc tgg ctt gac aag aac aag gac ccc ctg aat gag act gtg   1824
Ile Ala Gly Trp Leu Asp Lys Asn Lys Asp Pro Leu Asn Glu Thr Val
                595              600              605

gtg ggg ctg tac cag aag tct gca atg aag act ctg gct ctc ctc ttt   1872
Val Gly Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Leu Leu Phe
      610              615              620

att ggg gca acg gga gcg gaa gca gag gct ggc ggt gga aag aaa ggt   1920
Val Gly Ala Thr Gly Ala Glu Ala Glu Ala Gly Gly Gly Lys Lys Gly
625              630              635              640

ggt aag aag aag ggt tct tct ttc cag act gtg tcg gct ctc ttc agg   1968
Gly Lys Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu Phe Arg
                645              650              655

gag aat ttg aat aag ctg atg acc aac ttg agg agc act cac ccc cac   2016
Glu Asn Leu Asn Lys Leu Met Thr Asn Leu Arg Ser Thr His Pro His
                660              665              670

ttt gtg cgg tgc atc atc ccc aat gaa act aaa act cct ggt gcc atg   2064
Phe Val Arg Cys Ile Ile Pro Asn Glu Thr Lys Thr Pro Gly Ala Met
                675              680              685

gag cat gag att gtc ctg cat cag ctg agg tgt aac ggt gtg ctg gaa   2112
Glu His Glu Ile Val Leu His Gln Leu Arg Cys Asn Gly Val Leu Glu
```

690 695 700

ggc atc cgc atc tgc agg aaa ggc ttc cca agc aga atc ctt tat gca   2160
Gly Ile Arg Ile Cys Arg Lys Gly Phe Pro Ser Arg Ile Leu Tyr Ala
705   710   715   720

gac ttc aaa cag aga tac aag gtg tta aat gca agt gct atc cct gaa   2208
Asp Phe Lys Gln Arg Tyr Lys Val Leu Asn Ala Ser Ala Ile Pro Glu
            725   730   735

gga caa ttc atc gat agc aag aag gct tca gag aag ctc ctg ggg tcc   2256
Gly Gln Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu Gly Ser
            740   745   750

att gac att gac cac acc cag tat aaa ttt ggt cac acc aag gtc ttt   2304
Ile Asp Ile Asp His Thr Gln Tyr Lys Phe Gly His Thr Lys Val Phe
        755   760   765

ttc aaa gct ggt ctt ctg ggg ctc cta gag gag atg cga gat gag aag   2352
Phe Lys Ala Gly Leu Leu Gly Leu Leu Glu Glu Met Arg Asp Glu Lys
    770   775   780

ctg gcc cag ctg att acc cga acc cag gcc atg tgc aga ggg ttc ttg   2400
Leu Ala Gln Leu Ile Thr Arg Thr Gln Ala Met Cys Arg Gly Phe Leu
785   790   795   800

gca aga gtg gag tac cag aaa atg gtg gaa aga aga gag tcc atc ttc   2448
Ala Arg Val Glu Tyr Gln Lys Met Val Glu Arg Arg Glu Ser Ile Phe
            805   810   815

tgc atc cag tac aat gtc cgt gcc ttc atg aat gtc aag cac tgg ccc   2496
Cys Ile Gln Tyr Asn Val Arg Ala Phe Met Asn Val Lys His Trp Pro
            820   825   830

tgg atg aag ctg tat ttc aag atc aaa ccc ctc ctc aaa agt gca gag   2544
Trp Met Lys Leu Tyr Phe Lys Ile Lys Pro Leu Leu Lys Ser Ala Glu

          835   840   845

aca gag aag gag atg gcc aac atg aag gaa gaa ttt gag aaa acc aaa   2592
Thr Glu Lys Glu Met Ala Asn Met Lys Glu Glu Phe Glu Lys Thr Lys
     850   855   860

gaa gag ctg gct aag acc gag gca aaa agg aaa gag ctg gaa gaa aaa   2640
Glu Glu Leu Ala Lys Thr Glu Ala Lys Arg Lys Glu Leu Glu Glu Lys
865   870   875   880

atg gtg act ctg atg caa gaa aaa aat gac ttg caa ctc cag gtt caa   2688
Met Val Thr Leu Met Gln Glu Lys Asn Asp Leu Gln Leu Gln Val Gln
                885   890   895

gct gaa gct gac agc ttg gct gat gca gag gaa agg tgt gac cag cta   2736
Ala Glu Ala Asp Ser Leu Ala Asp Ala Glu Glu Arg Cys Asp Gln Leu
          900   905   910

atc aaa acc aaa atc cag cta gaa gcc aaa atc aaa gag gtg act gag   2784
Ile Lys Thr Lys Ile Gln Leu Glu Ala Lys Ile Lys Glu Val Thr Glu
     915   920   925

aga gct gag gat gag gaa gag atc aat gct gag ctg aca gcc aag aag   2832
Arg Ala Glu Asp Glu Glu Glu Ile Asn Ala Glu Leu Thr Ala Lys Lys
930   935   940

agg aaa ctg gag gat gaa tgt tca gaa ctc aag aaa gac att gat gac   2880
Arg Lys Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile Asp Asp
945   950   955   960

ctt gag ctg aca ctg gcc aag gtt gag aag gag aaa cat gcc aca gaa   2928
Leu Glu Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala Thr Glu
                965   970   975

aac aag gtg aaa aac ctc aca gaa gag atg gcg ggt ctg gat gaa acc   2976
Asn Lys Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp Glu Thr

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               980              985              990

att gct aag ctg acc aag gag aag  aag gct ctc cag gag  gcc cac cag   3024
Ile Ala Lys Leu Thr Lys Glu Lys  Lys Ala Leu Gln Glu  Ala His Gln
         995              1000              1005

cag acc  ctg gat gac ctg cag  gca gag gag gac aaa  gtc aac acc      3069
Gln Thr  Leu Asp Asp Leu Gln  Ala Glu Glu Asp Lys  Val Asn Thr
         1010              1015              1020

ctg acc  aaa gct aaa atc aaa  ctt gaa caa caa gtg  gat gat ctt      3114
Leu Thr  Lys Ala Lys Ile Lys  Leu Glu Gln Gln Val  Asp Asp Leu
         1025              1030              1035

gaa gga  tct ttg gaa caa gaa  aag aaa atc cgg atg  gat cta gaa      3159
Glu Gly  Ser Leu Glu Gln Glu  Lys Lys Ile Arg Met  Asp Leu Glu
         1040              1045              1050

aga gca  aag aga aaa cta gag  gga gac cta aaa ttg  gct caa gaa      3204
Arg Ala  Lys Arg Lys Leu Glu  Gly Asp Leu Lys Leu  Ala Gln Glu
         1055              1060              1065

tcc gca  atg gat ata gaa aat  gac aaa caa caa ctt  gat gaa aag      3249
Ser Ala  Met Asp Ile Glu Asn  Asp Lys Gln Gln Leu  Asp Glu Lys
         1070              1075              1080

ctt aaa  aag aaa gag ttt gaa  atg agc ggt ctg caa  agc aag att      3294
Leu Lys  Lys Lys Glu Phe Glu  Met Ser Gly Leu Gln  Ser Lys Ile
         1085              1090              1095

gaa gat  gaa caa gcc ctt ggt  atg cag ctg cag aag  aaa atc aag      3339
Glu Asp  Glu Gln Ala Leu Gly  Met Gln Leu Gln Lys  Lys Ile Lys
         1100              1105              1110

gag tta  caa gcc cgc att gag  gaa ctg gag gag gaa  atc gag gca      3384
Glu Leu  Gln Ala Arg Ile Glu  Glu Leu Glu Glu Glu  Ile Glu Ala
```

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      1115                  1120                  1125

gag cgg  gcc tcc cgg gcc aaa  gca gag aag cag agc  tct gat ctc    3429
Glu Arg  Ala Ser Arg Ala Lys  Ala Glu Lys Gln Arg  Ser Asp Leu
     1130                 1135                 1140


tcc cgg  gag ctg gag gag atc  agt gag agg ctg gaa  gaa gcc ggt    3474
Ser Arg  Glu Leu Glu Glu Ile  Ser Glu Arg Leu Glu  Glu Ala Gly
    1145                 1150                 1155


ggg gcc  acc tcg gcc cag att  gag atg aac aag aag  cgg gaa gct    3519
Gly Ala  Thr Ser Ala Gln Ile  Glu Met Asn Lys Lys  Arg Glu Ala
    1160                 1165                 1170


gag ttc  cag aaa atg cgc agg  gac ctg gag gag gcc  acc cta cag    3564
Glu Phe  Gln Lys Met Arg Arg  Asp Leu Glu Glu Ala  Thr Leu Gln
    1175                 1180                 1185


cat gag  gcc acg gcg gcc acc  ctg agg aag aag cat  gca gat agt    3609
His Glu  Ala Thr Ala Ala Thr  Leu Arg Lys Lys His  Ala Asp Ser
    1190                 1195                 1200


gtg gcc  gag ctt ggg gag cag  att gac aac ctg cag  cga gtg aag    3654
Val Ala  Glu Leu Gly Glu Gln  Ile Asp Asn Leu Gln  Arg Val Lys
    1205                 1210                 1215


cag aag  ctg gag aag gag aag  agt gag atg aag atg  gag atc gat    3699
Gln Lys  Leu Glu Lys Glu Lys  Ser Glu Met Lys Met  Glu Ile Asp
    1220                 1225                 1230


gac ctt  gct agt aac atg gag  act gtc tcc aaa gcc  aag gga aac    3744
Asp Leu  Ala Ser Asn Met Glu  Thr Val Ser Lys Ala  Lys Gly Asn
    1235                 1240                 1245


ctt gaa  aag atg tgc cgc gct  cta gaa gat cag ctg  agt gaa att    3789
Leu Glu  Lys Met Cys Arg Ala  Leu Glu Asp Gln Leu  Ser Glu Ile
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          1250                1255                1260
aag acc   aag gaa gag gag cag   cag cgg ctg atc aat   gac ctc aca      3834
Lys Thr   Lys Glu Glu Glu Gln   Gln Arg Leu Ile Asn   Asp Leu Thr
          1265                1270                1275

gca cag   aga gcg cgc ctg caa   aca gaa tca ggt gaa   tat tca cgc      3879
Ala Gln   Arg Ala Arg Leu Gln   Thr Glu Ser Gly Glu   Tyr Ser Arg
          1280                1285                1290

cag cta   gat gaa aag gac aca   cta gtt tca cag ctc   tcg agg ggc      3924
Gln Leu   Asp Glu Lys Asp Thr   Leu Val Ser Gln Leu   Ser Arg Gly
          1295                1300                1305

aaa caa   gcc ttt act caa cag   att gag gaa ctg aaa   agg caa ctt      3969
Lys Gln   Ala Phe Thr Gln Gln   Ile Glu Glu Leu Lys   Arg Gln Leu
          1310                1315                1320

gaa gag   gag ata aag gcc aag   agt gcc ctg gca cat   gcc ctg cag      4014
Glu Glu   Glu Ile Lys Ala Lys   Ser Ala Leu Ala His   Ala Leu Gln
          1325                1330                1335

tcc tcc   cgc cat gac tgt gac   ctg ctg cgg gaa cag   tat gag gag      4059
Ser Ser   Arg His Asp Cys Asp   Leu Leu Arg Glu Gln   Tyr Glu Glu
          1340                1345                1350

gag cag   gaa gcc aag gcc gag   cta cag aga gca atg   tcc aag gcc      4104
Glu Gln   Glu Ala Lys Ala Glu   Leu Gln Arg Ala Met   Ser Lys Ala
          1355                1360                1365

aac agt   gag gtt gcc cag tgg   agg acc aaa tat gag   aca gat gcc      4149
Asn Ser   Glu Val Ala Gln Trp   Arg Thr Lys Tyr Glu   Thr Asp Ala
          1370                1375                1380

atc cag   cgc aca gag gag ctg   gag gag gcc aag aag   aag ctg gct      4194
Ile Gln   Arg Thr Glu Glu Leu   Glu Glu Ala Lys Lys   Lys Leu Ala
```

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       1385              1390              1395

cag cgt  ctg cag gat gct gag  gaa cat gta gaa gct  gtg aat gcc    4239
Gln Arg  Leu Gln Asp Ala Glu  Glu His Val Glu Ala  Val Asn Ala
     1400              1405              1410

aaa tgt  gct tcc ctt gag aag  acg aag cag agg ctc  cag aat gaa    4284
Lys Cys  Ala Ser Leu Glu Lys  Thr Lys Gln Arg Leu  Gln Asn Glu
     1415              1420              1425

gtt gag  gac ctc atg att gat  gtt gag agg aca aat  gct gcc tgt    4329
Val Glu  Asp Leu Met Ile Asp  Val Glu Arg Thr Asn  Ala Ala Cys
     1430              1435              1440

gcc gcc  ctg gac aaa aag caa  agg aac ttt gat aag  atc ctg gca    4374
Ala Ala  Leu Asp Lys Lys Gln  Arg Asn Phe Asp Lys  Ile Leu Ala
     1445              1450              1455

gaa tgg  aaa cag aag tgt gaa  gaa act cat gct gaa  ctt gaa gct    4419
Glu Trp  Lys Gln Lys Cys Glu  Glu Thr His Ala Glu  Leu Glu Ala
     1460              1465              1470

tct caa  aag gaa tcc cgc tca  ctc agc aca gaa cta  ttt aag att    4464
Ser Gln  Lys Glu Ser Arg Ser  Leu Ser Thr Glu Leu  Phe Lys Ile
     1475              1480              1485

aag aat  gct tat gag gaa tct  tta gac caa ctt gaa  acc ttg aaa    4509
Lys Asn  Ala Tyr Glu Glu Ser  Leu Asp Gln Leu Glu  Thr Leu Lys
     1490              1495              1500

cgg gaa  aat aag aat ctg cag  cag gag att tct gat  ctc act gaa    4554
Arg Glu  Asn Lys Asn Leu Gln  Gln Glu Ile Ser Asp  Leu Thr Glu
     1505              1510              1515

cag att  gca gaa gga gga aag  cgc atc cat gaa ctg  gaa aaa ata    4599
Gln Ile  Ala Glu Gly Gly Lys  Arg Ile His Glu Leu  Glu Lys Ile
```

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      1520                  1525                 1530

aag aag  caa gtt gag caa gaa  aag tct gaa ctt cag  gct gcc tta      4644
Lys Lys  Gln Val Glu Gln Glu  Lys Ser Glu Leu Gln  Ala Ala Leu
      1535                  1540                 1545


gag gag  gca gag gca tct ctt  gaa cat gaa gag aga  aag atc ctg      4689
Glu Glu  Ala Glu Ala Ser Leu  Glu His Glu Glu Gly  Lys Ile Leu
      1550                  1555                 1560


cgc atc  cag ctt gag ttg aac  caa gtc aag tct gag  gtt gat agg      4734
Arg Ile  Gln Leu Glu Leu Asn  Gln Val Lys Ser Glu  Val Asp Arg
      1565                  1570                 1575


aaa att  gct gaa aaa gat gag  gaa att gac cag atg  aag aga aac      4779
Lys Ile  Ala Glu Lys Asp Glu  Glu Ile Asp Gln Met  Lys Arg Asn
      1580                  1585                 1590


cac att  aga atc gtg gag tcc  atg cag agc aca ctg  gat gct gag      4824
His Ile  Arg Ile Val Glu Ser  Met Gln Ser Thr Leu  Asp Ala Glu
      1595                  1600                 1605


atc agg  agc agg aat gat gcc  att agg ctc aag aag  aag atg gag      4869
Ile Arg  Ser Arg Asn Asp Ala  Ile Arg Leu Lys Lys  Lys Met Glu
      1610                  1615                 1620


gga gac  cte aat gaa atg gaa  atc cag ctg aac cat  gcc aac cgc      4914
Gly Asp  Leu Asn Glu Met Glu  Ile Gln Leu Asn His  Ala Asn Arg
      1625                  1630                 1635


atg gct  gct gag gcc ctg agg  aac tat agg aac acc  caa gcc atc      4959
Met Ala  Ala Glu Ala Leu Arg  Asn Tyr Arg Asn Thr  Gln Ala Ile
      1640                  1645                 1650


ctc aag  gat acc cag ctc cac  cta gat gat gct ctc  cgg agc caa      5004
Leu Lys  Asp Thr Gln Leu His  Leu Asp Asp Ala Leu  Arg Ser Gln
```

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      1655              1660              1665


gag gac  ctg aag gaa cag ctg  gct atg gtg gag cgc  aga gcc aac      5049
Glu Asp  Leu Lys Glu Gln Leu  Ala Met Val Glu Arg  Arg Ala Asn
     1670              1675              1680


ctg ctg  cag gct gag atc gag  gaa cta cga gcc act  ctg gaa cag      5094
Leu Leu  Gln Ala Glu Ile Glu  Glu Leu Arg Ala Thr  Leu Glu Gln
     1685              1690              1695


acg gag  agg agc aaa atc  gca gaa cag gag ctc  ctg gat gcc          5139
Thr Glu  Arg Ser Lys Ile  Ala Glu Gln Glu Leu  Leu Asp Ala
     1700              1705              1710


agt gaa  cgt gtt cag ctc ctg  cac acc cag aac acc  agc ctg atc      5184
Ser Glu  Arg Val Gln Leu Leu  His Thr Gln Asn Thr  Ser Leu Ile
     1715              1720              1725


aac acc  aag aag aag ctg gag  aca gac att tcc caa  atc cag gga      5229
Asn Thr  Lys Lys Lys Leu Glu  Thr Asp Ile Ser Gln  Ile Gln Gly
     1730              1735              1740


gag atg  gaa gac atc atc cag  gaa gcc cgc aat gca  gaa gag aag      5274
Glu Met  Glu Asp Ile Ile Gln  Glu Ala Arg Asn Ala  Glu Glu Lys
     1745              1750              1755


gcc aag  aag gcc atc act gat  gct gcc atg atg gct  gag gag ctg      5319
Ala Lys  Lys Ala Ile Thr Asp  Ala Ala Met Met Ala  Glu Glu Leu
     1760              1765              1770


aag aag  gaa cag gac acc agc  gcc cat ctg gag cgg  atg aag aag      5364
Lys Lys  Glu Gln Asp Thr Ser  Ala His Leu Glu Arg  Met Lys Lys
     1775              1780              1785


aac ttg  gaa cag acg gtg aag  gac ctg cag cat cgt  ctg gat gag      5409
Asn Leu  Glu Gln Thr Val Lys  Asp Leu Gln His Arg  Leu Asp Glu
```

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        1790                  1795                  1800
gct gag  cag ctg gcc ctg aag  ggt ggg aag aag cag  atc cag aaa    5454
Ala Glu  Gln Leu Ala Leu Lys  Gly Gly Lys Lys Gln  Ile Gln Lys
     1805                  1810                  1815

ctg gag  gcc agg gtt cgt gaa  ctt gaa ggt gaa gtt  gaa agt gaa    5499
Leu Glu  Ala Arg Val Arg Glu  Leu Glu Gly Glu Val  Glu Ser Glu
     1820                  1825                  1830

cag aag  cgc aat gtt gaa gct  gtc aag ggt cta cgc  aaa cat gag    5544
Gln Lys  Arg Asn Val Glu Ala  Val Lys Gly Leu Arg  Lys His Glu
     1835                  1840                  1845

aga aaa  gtg aag gaa ctc act  tac caa act gag gaa  gac cgc aag    5589
Arg Lys  Val Lys Glu Leu Thr  Tyr Gln Thr Glu Glu  Asp Arg Lys
     1850                  1855                  1860

aat att  ctc agg ctg cag gac  ctg gtg gac aag ctg  caa gca aag    5634
Asn Ile  Leu Arg Leu Gln Asp  Leu Val Asp Lys Leu  Gln Ala Lys
     1865                  1870                  1875

gtg aaa  tcc tac aag aga caa  gct gaa gaa gcg gag  gaa caa tcc    5679
Val Lys  Ser Tyr Lys Arg Gln  Ala Glu Glu Ala Glu  Glu Gln Ser
     1880                  1885                  1890

aac gtc  aac ctc tcc aaa ttc  cgg agg atc cag cac  gag ctg gag    5724
Asn Val  Asn Leu Ser Lys Phe  Arg Arg Ile Gln His  Glu Leu Glu
     1895                  1900                  1905

gag gcc  gag gaa agg gct gac  att gct gag tcc cag  gtc aac aag    5769
Glu Ala  Glu Glu Arg Ala Asp  Ile Ala Glu Ser Gln  Val Asn Lys
     1910                  1915                  1920

ctg agg  gtg aag agc agg gag  gtt cac aca aaa atc  ata agt gaa    5814
Leu Arg  Val Lys Ser Arg Glu  Val His Thr Lys Ile  Ile Ser Glu
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    1925   1930   1935

gag taa tttatctaac tgctgaaagg tgaccaaaga aatgcacaaa atgtgaaaat   5870
Glu

ctttgtcact ccattttgta cttstgactt ttggagataa aaaattiatc tgcca   5925

<210> 6
<211> 1839
<212> PRT
<213> Homo sapiens

<400> 6

Met Ser Ser Asp Ser Glu Met Ala Ile Phe Gly Glu Ala Ala Pro Phe
1   5   10   15

Leu Arg Lys Ser Glu Arg Glu Arg Ile Glu Ala Gln Asn Lys Pro Phe
             20   25   30

Asp Ala Lys Thr Ser Val Phe Val Val Asp Pro Lys Glu Ser Phe Val
         35   40   45

Lys Ala Thr Val Gln Ser Arg Glu Gly Gly Lys Val Thr Ala Lys Thr
     50   55   60

Glu Ala Gly Ala Thr Val Thr Val Lys Asp Asp Gln Val Phe Pro Met
65   70   75   80

Asn Pro Pro Lys Tyr Asp Lys Ile Glu Asp Met Ala Met Met Thr His
                 85   90   95

Leu His Gle Pro Ala Val Leu Tyr Asn Leu Lys Glu Arg Tyr Ala Ala
                 100   105   110

Trp Met Ile Tyr Thr Tyr Ser Gly Leu Phe Cys Val Thr Val Asn Pro
                 115   120   125

Tyr Lys Trp Leu Pro Val Tyr Asn Ala Glu Val Val Thr Ala Tyr Arg
      130   135   140

Gly Lys Lys Arg Gln Glu Ala Pro Pro His Ile Phe Ser Ile Ser Asp
145   150   155   160

Asn Ala Tyr Gln Phe Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu
                 165   170   175

Ile Thr Gly Glu Ser Gly Ala Gly Lys Thr Val Asn Thr Lys Arg Val
                 180   185   190

Ile Gln Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu
      195   200   205

Glu Val Thr Ser Gly Lys Met Gln Gly Thr Leu Glu Asp Gln Ile Ile
      210   215   220

Ser Ala Asn Pro Leu Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg
225   230   235   240

Asn Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly
                245   250   255

Thr Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu
                260   265   270

Lys Ser Arg Val Thr Phe Gln Leu Lys Ala Glu Arg Ser Tyr His Ile
        275   280   285

Phe Tyr Gln Ile Met Ser Asn Lys Lys Pro Asp Leu Ile Glu Met Leu
        290   295   300

Leu Ile Thr Thr Asn Pro Tyr Asp Tyr Ala Phe Val Ser Gln Gly Glu
305   310   315   320

Ile Thr Val Pro Ser Ile Asp Asp Gln Glu Glu Leu Met Ala Thr Asp
                325   330   335

Ser Ala Ile Glu Ile Leu Gly Phe Thr Ser Asp Glu Arg Val Ser Ile
        340   345   350

Tyr Lys Leu Thr Gly Ala Val Met His Tyr Gly Asn Met Lys Phe Lys
        355   360   365

Gln Lys Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Val Ala
    370   375   380

Asp Lys Ala Ala Tyr Leu Gln Asn Leu Asn Ser Ala Asp Leu Leu Lys
385   390   395   400

Ala Leu Cys Tyr Pro Arg Val Lys Val Gly Asn Glu Tyr Val Thr Lys
        405   410   415

Gly Gln Thr Val Gln Gln Val Tyr Asn Ala Val Gly Ala Leu Ala Lys
        420   425   430

Ala Val Tyr Asp Lys Met Phe Leu Trp Met Val Thr Arg Ile Asn Gln
      435   440   445

Gln Leu Asp Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp
    450   455   460

Ile Ala Gly Phe Glu Ile Phe Asp Phe Asn Ser Leu Glu Gln Leu Cys
465   470   475   480

Ile Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met
        485   490   495

Phe Val Leu Glu Gln Glu Glu Tyr Lys Lys Glu Gly Ile Glu Trp Thr
        500   505   510

Phe Ile Asp Phe Gly Met Asp Leu Ala Ala Cys Ile Glu Leu Ile Glu
        515   520   525

Lys Pro Met Gly Ile Phe Ser Ile Leu Glu Glu Glu Cys Met Phe Pro
        530   535   540

Lys Ala Thr Asp Thr Ser Phe Lys Asn Lys Leu Tyr Glu Gln His Leu
545   550   555   560

Gly Lys Ser Asn Asn Phe Gln Lys Pro Lys Pro Ala Lys Gly Lys Pro
                565   570   575

Glu Ala His Phe Ser Leu Ile His Tyr Ala Gly Thr Val Asp Tyr Asn
                580   585   590

Ile Ala Gly Trp Leu Asp Lys Asn Lys Asp Pro Leu Asn Glu Thr Val
                595   600   605

Val Gly Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Leu Leu Phe
        610   615   620

Val Gly Ala Thr Gly Ala Glu Ala Glu Ala Gly Gly Gly Lys Lys Gly
625   630   635   640

Gly Lys Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu Phe Arg
                645   650   655

Glu Asn Leu Asn Lys Leu Met Thr Asn Leu Arg Ser Thr His Pro His
            660   665   670

Phe Val Arg Cys Ile Ile Pro Asn Glu Thr Lys Thr Pro Gly Ala Met
            675   680   685

Glu His Glu Leu Val Leu His Gln Leu Arg Cys Asn Gly Val Leu Glu
        690   695   700

Gly Ile Arg Ile Cys Arg Lys Gly Phe Pro Ser Arg Ile Leu Tyr Ala
705   710   715   720

Asp Phe Lys Gln Arg Tyr Lys Val Leu Asn Ala Ser Ala Ile Pro Glu
                725   730   735

Gly Gln Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu Gly Ser
            740   745   750

Ile Asp Ile Asp His Thr Gln Tyr Lys Phe Gly His Thr Lys Val Phe
            755   760   765

Phe Lys Ala Gly Leu Leu Gly Leu Leu Glu Glu Met Arg Asp Glu Lys
        770   775   780

Leu Ala Gln Leu Ile Thr Arg Thr Gln Ala Met Cys Arg Gly Phe Leu
785   790   795   800

Ala Arg Val Glu Tyr Gln Lys Met Val Glu Arg Arg Glu Ser Ile Phe
          805   810   815

Cys Ile Gln Tyr Asn Val Arg Ala Phe Met Asn Val Lys His Trp Pro
        820   825   830

Trp Met Lys Leu Tyr Phe Lys Ile Lys Pro Leu Leu Lys Ser Ala Glu
        835   840   845

Thr Glu Lys Glu Met Ala Asn Met Lys Glu Glu Phe Glu Lys Thr Lys
      850   855   860

Glu Glu Leu Ala Lys Thr Glu Ala Lys Arg Lys Glu Leu Glu Glu Lys
865   870   875   880

Met Val Thr Leu Met Gln Glu Lys Asn Asp Leu Gln Leu Gln Val Gln
        885   890   895

Ala Glu Ala Asp Ser Leu Ala Asp Ala Glu Glu Arg Cys Asp Gln Leu
        900   905   910

Ile Lys Thr Lys Ile Gln Leu Glu Ala Lys Ile Lys Glu Val Thr Glu
        915   920   925

Arg Ala Glu Asp Glu Glu Glu Ile Asn Ala Glu Leu Thr Ala Lys Lys
     930   935   940

Arg Lys Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile Asp Asp
945   950   955   960

Leu Glu Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala Thr Glu
                965   970   975

Asn Lys Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp Glu Thr
             980   985   990

Ile Ala Lys Leu Thr Lys Glu Lys Lys Ala Leu Gln Glu Ala His Gln
             995   1000   1005

Gln Thr Leu Asp Asp Leu Gln Ala Glu Glu Asp Lys Val Asn Thr
     1010   1015   1020

Leu Thr Lys Ala Lys Ile Lys Leu Glu Gln Gln Val Asp Asp Leu
     1025   1030   1035

Glu Gly Ser Leu Glu Gln Glu Lys Lys Ile Arg Met Asp Leu Glu
     1040   1045   1050

Arg Ala Lys Arg Lys Leu Glu Gly Asp Leu Lys Leu Ala Gln Glu
     1055   1060   1065

Ser Ala Met Asp Ile Glu Asn Asp Lys Gln Gln Leu Asp Glu Lys
     1070   1075   1080

Leu Lys Lys Lys Glu Phe Glu Met Ser Gly Leu Gln Ser Lys Ile
    1085   1090   1095

Glu Asp Glu Gln Ala Leu Gly Met Gln Leu Gln Lys Lys Ile Lys
    1100   1105   1110

Glu Leu Gln Ala Arg Ile Glu Glu Leu Glu Glu Glu Ile Glu Ala
    1115   1120   1125

Glu Arg Ala Ser Arg Ala Lys Ala Glu Lys Gln Arg Ser Asp Leu
    1130   1135   1140

Ser Arg Glu Leu Glu Glu Ile Ser Glu Arg Leu Glu Glu Ala Gly
    1145   1150   1155

Gly Ala Thr Ser Ala Gln Ile Glu Met Asn Lys Lys Arg Glu Ala
    1160   1165   1170

Glu Phe Gln Lys Met Arg Arg Asp Leu Glu Glu Ala Thr Leu Gln
    1175   1180   1185

His Glu Ala Thr Ala Ala Thr Leu Arg Lys Lys His Ala Asp Ser
    1190   1195   1200

Val Ala Glu Leu Gly Glu Gln Ile Asp Asn Leu Gln Arg Val Lys
    1205   1210   1215

Gln Lys Leu Glu Lys Glu Lys Ser Glu Met Lys Met Glu Ile Asp
    1220   1225   1230

Asp Leu Ala Ser Asn Met Glu Thr Val Ser Lys Ala Lys Gly Asn
    1235   1240   1245

Leu Glu Lys Met Cys Arg Ala Leu Glu Asp Gln Leu Ser Glu Ile
    1250   1255   1260

Lys Thr Lys Glu Glu Glu Gln Gln Arg Leu Ile Asn Asp Leu Thr
    1265   1270   1275

Ala Gln Arg Ala Arg Leu Gln Thr Glu Ser Gly Glu Tyr Ser Arg
    1280   1285   1290

Gln Leu Asp Glu Lys Asp Thr Leu Val Ser Gln Leu Ser Arg Gly
    1295   1300   1305

Lys Gln Ala Phe Thr Gln Gln Ile Glu Glu Leu Lys Arg Gln Leu
    1310   1315   1320

Glu Glu Glu Ile Lys Ala Lys Ser Ala Leu Ala His Ala Leu Gln
    1325   1330   1335

Ser Ser Arg His Asp Cys Asp Leu Leu Arg Glu Gln Tyr Glu Glu
    1340   1345   1350

Glu Gln Glu Ala Lys Ala Glu Leu Gln Arg Ala Met Ser Lys Ala
    1355   1360   1365

Asn Ser Glu Val Ala Gln Trp Arg Thr Lys Tyr Glu Thr Asp Ala
    1370   1375   1380

Ile Gln Arg Thr Glu Glu Leu Glu Glu Ala Lys Lys Lys Leu Ala
    1385   1390   1395

Gln Arg Leu Gln Asp Ala Glu Glu His Val Glu Ala Val Asn Ala
    1400   1405   1410

Lys Cys Ala Ser Leu Glu Lys Thr Lys Gln Arg Leu Gln Asn Glu
    1415   1420   1425

Val Glu Asp Leu Met Ile Asp Val Glu Arg Thr Asn Ala Ala Cys
    1430   1435   1440

Ala Ala Leu Asp Lys Lys Gln Arg Asn Phe Asp Lys Ile Leu Ala
    1445   1450   1455

Glu Trp Lys Gln Lys Cys Glu Glu Thr His Ala Glu Leu Glu Ala
    1460   1465   1470

Ser Gln Lys Glu Ser Arg Ser Leu Ser Thr Glu Leu Phe Lys Ile
    1475   1480   1485

Lys Asn Ala Tyr Glu Glu Ser Leu Asp Gln Leu Glu Thr Leu Lys
1490   1495   1500

Arg Glu Asn Lys Asn Leu Gln Gln Glu Ile Ser Asp Leu Thr Glu
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Gln Ile Ala Glu Gly Gly Lys Arg Ile His Glu Leu Glu Lys Ile
1520   1525   1530

Lys Lys Gln Val Glu Gln Glu Lys Ser Glu Leu Gln Ala Ala Leu
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Glu Glu Ala Glu Ala Ser Leu Glu His Glu Glu Gly Lys Ile Leu
1550   1555   1560

Arg Ile Gln Leu Glu Leu Asn Gln Val Lys Ser Glu Val Asp Arg
1565   1570   1575

Lys Ile Ala Glu Lys Asp Glu Glu Ile Asp Gln Met Lys Arg Asn
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His Ile Arg Ile Val Glu Ser Met Gln Ser Thr Leu Asp Ala Glu
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Ile Arg Ser Arg Asn Asp Ala Ile Arg Leu Lys Lys Lys Met Glu
1610   1615   1620

Gly Asp Leu Asn Glu Met Glu Ile Gln Leu Asn His Ala Asn Arg
    1625   1630   1635

Met Ala Ala Glu Ala Leu Arg Asn Tyr Arg Asn Thr Gln Ala Ile
    1640   1645   1650

Leu Lys Asp Thr Gln Leu His Leu Asp Asp Ala Leu Arg Ser Gln
    1655   1660   1665

Glu Asp Leu Lys Glu Gln Leu Ala Met Val Glu Arg Arg Ala Asn
    1670   1675   1680

Leu Leu Gln Ala Glu Ile Glu Glu Leu Arg Ala Thr Leu Glu Gln
    1685   1690   1695

Thr Glu Arg Ser Arg Lys Ile Ala Glu Gln Glu Leu Leu Asp Ala
    1700   1705   1710

Ser Glu Arg Val Gln Leu Leu His Thr Gln Asn Thr Ser Leu Ile
    1715   1720   1725

Asn Thr Lys Lys Lys Leu Glu Thr Asp Ile Ser Gln Ile Gln Gly
    1730   1735   1740

Glu Met Glu Asp Ile Ile Gln Glu Ala Arg Asn Ala Glu Glu Lys
    1745   1750   1755

Ala Lys Lys Ala Ile Thr Asp Ala Ala Met Met Ala Gln Glu Leu
     1760   1765   1770

Lys Lys Glu Gln Asp Thr Ser Ala His Leu Glu Arg Met Lys Lys
     1775   1780   1785

Asn Leu Glu Gln Thr Val Lys Asp Leu Gln His Arg Leu Asp Glu
     1790   1795   1800

Ala Glu Gln Leu Ala Leu Lys Gly Gly Lys Lys Gln Ile Gln Lys
     1805   1810   1815

Leu Glu Ala Arg Val Arg Glu Leu Glu Gly Glu Val Glu Ser Glu
     1820   1825   1830

Gln Lys Arg Asn Val Glu Ala Val Lys Gly Leu Arg Lys His Glu
     1835   1840   1845

Arg Lys Val Lys Glu Leu Thr Tyr Gln Thr Glu Glu Asp Arg Lys
     1850   1855   1860

Asn Ile Leu Arg Leu Gln Asp Leu Val Asp Lys Leu Gln Ala Lys
     1865   1870   1875

Val Lys Ser Tyr Lys Arg Gln Ala Glu Glu Ala Glu Glu Gln Ser
     1880   1885   1890

Asn Val Asn Leu Ser Lys Phe Arg Arg Ile Gln His Glu Leu Glu
    1895   1900   1905

Glu Ala Glu Glu Arg Ala Asp Ile Ala Glu Ser Gln Val Asn Lys
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Leu Arg Val Lys Ser Arg Glu Val His Thr Lys Ile Ile Ser Glu
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Glu

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Leu Ile Trp Thr Leu Phe Phe Leu Gly Thr Ala Val Ser Leu Gln Val
              10   15   20

gat att gtt ccc agc cag ggg gag atc agc gtt gga gag tcc aaa ttc   151

Asp Ile Val Pro Ser Gln Gly Glu Ile Ser Val Gly Glu Ser Lys Phe
　　　　　25　　　　　　　　　30　　　　　　　　　35

ttc tta tgc caa gtg gca gga gat gcc aaa gat aaa gac atc tcc tgg　199
Phe Leu Cys Gln Val Ala Gly Asp Ala Lys Asp Lys Asp Ile Ser Trp
　40　　　　　　　　　45　　　　　　　　　50

ttc tcc ccc aat gga gaa aag ctc acc cca aac cag cag cgg atc tca　247
Phe Ser Pro Asn Gly Glu Lys Leu Thr Pro Asn Gln Gln Arg Ile Ser
55　　　　　　　　　60　　　　　　　　　65　　　　　　　　　70

gtg gtg tgg aat gat gat tcc tcc tcc acc ctc acc atc tat aac gcc　295
Val Val Trp Asn Asp Asp Ser Ser Ser Thr Leu Thr Ile Tyr Asn Ala
　　　　　　　　　75　　　　　　　　　80　　　　　　　　　85

aac atc gac gac gcc ggc att tac aag tgt gtg gtt aca ggc gag gat　343
Asn Ile Asp Asp Ala Gly Ile Tyr Lys Cys Val Val Thr Gly Glu Asp
　　　　　　　　　90　　　　　　　　　95　　　　　　　　　100

ggc agt gag tca gag gcc acc gtc aac gtg aag atc ttt cag aag ctc　391
Gly Ser Glu Ser Glu Ala Thr Val Asn Val Lys Ile Phe Gln Lys Leu
　　　　　105　　　　　　　　　110　　　　　　　　　115

atg ttc aag aat gcg cca acc cca cag gag ttc cgg gag ggg gaa gat　439
Met Phe Lys Asn Ala Pro Thr Pro Gln Glu Phe Arg Glu Gly Glu Asp
　　　120　　　　　　　　　125　　　　　　　　　130

gcc gtg att gtg tgt gat gtg gtc agc tcc ctc cca ccc acc atc atc　487
Ala Val Ile Val Cys Asp Val Val Ser Ser Leu Pro Pro Thr Ile Ile
135　　　　　　　　　140　　　　　　　　　145　　　　　　　　　150

tgg aaa cac aaa ggc cgc gat gtc atc ctg aaa aaa gat gtc cga ttc　535
Trp Lys His Lys Gly Arg Asp Val Ile Leu Lys Lys Asp Val Arg Phe
　　　　　155　　　　　　　　　160　　　　　　　　　165

ata gtc ctg tcc aac aac tac ctg cag atc cgg ggc atc aag aaa aca　583

Ile Val Leu Ser Asn Asn Tyr Leu Gln Ile Arg Gly Ile Lys Lys Thr
            170   175   180

gat gag ggc act tat cgc tgt gag ggc aga atc ctg gca cgg ggg gag   631
Asp Glu Gly Thr Tyr Arg Cys Glu Gly Arg Ile Leu Ala Arg Gly Glu
            185   190   195

atc aac ttc aag gac att cag gtc att gtg aat gtg cca cct acc atc   679
Ile Asn Phe Lys Asp Ile Gln Val Ile Val Asn Val Pro Pro Thr Ile
        200   205   210

cgg gcc agg cag aat att gtg aat gcc acc gcc aac ctc ggc cag tcc   727
Arg Ala Arg Gln Asn Ile Val Asn Ala Thr Ala Asn Leu Gly Gln Ser
    215   220   225   230

gtc acc ctg gtg tgc gat gcc gaa cgg ttc cca gag ccc acc atg agc   775
Val Thr Leu Val Cys Asp Ala Glu Arg Phe Pro Glu Pro Thr Met Ser
                235   240   245

tgg aca aag gat ggg gaa cag ata gag cag gag gaa gac gat gag aag   823
Trp Thr Lys Asp Gly Glu Gln Ile Glu Gln Glu Glu Asp Asp Glu Lys
            250   255   260

tac atc ttc agc gac gat agt tcc cag ctg acc atc aaa aag gtg gat   871
Tyr Ile Phe Ser Asp Asp Ser Ser Gln Leu Thr Ile Lys Lys Val Asp
            265   270   275

aag aac gac gag gct gag tac atc tgc att gct gag aac aag gct ggc   919
Lys Asn Asp Glu Ala Glu Tyr Ile Cys Ile Ala Glu Asn Lys Ala Gly
        280   285   290

gag cag gat gcg acc atc cac ctc aaa gtc ttt gca aaa ccc aaa atc   967
Glu Gln Asp Ala Thr Ile His Leu Lys Val Phe Ala Lys Pro Lys Ile
    295   300   305   310

aca tat gta gag aac cag act gcc atg gaa tta gag gag cag gtc act   1015

Thr Tyr Val Glu Asn Gln Thr Ala Met Glu Leu Glu Glu Gln Val Thr
            315   320   325

ctt acc tgt gaa gcc tcc gga gac ccc att ccc tcc atc acc tgg agg   1063
Leu Thr Cys Glu Ala Ser Gly Asp Pro Ile Pro Ser Ile Thr Trp Arg
            330   335   340

act tct acc cgg aac atc agc agc gaa gag aag act ctg gat ggg cac   1111
Thr Ser Thr Arg Asn Ile Ser Ser Glu Glu Lys Thr Leu Asp Gly His
            345   350   355

atg gtg gtg cgt agc cat gcc cgt gtg tcg tcg ctg acc ctg aag agc   1159
Met Val Val Arg Ser His Ala Arg Val Ser Ser Leu Thr Leu Lys Ser
            360   365   370

atc cag tac act gat gcc gga gag tac atc tgc acc gcc agc aac acc   1207
Ile Gln Tyr Thr Asp Ala Gly Glu Tyr Ile Cys Thr Ala Ser Asn Thr
375   380   385   390

atc ggc cag gac tcc cag tcc atg tac ctt gaa gtg caa tat gcc cca   1255
Ile Gly Gln Asp Ser Gln Ser Met Tyr Leu Glu Val Gln Tyr Ala Pro
                395   400   405

aag cta cag ggc cct gtg gct gtg tac act tgg gag ggg aac cag gtg   1303
Lys Leu Gln Gly Pro Val Ala Val Tyr Thr Trp Glu Gly Asn Gln Val
            410   415   420

aac atc acc tgc gag gta ttt gcc tat ccc agt gcc acg atc tcc tgg   1351
Asn Ile Thr Cys Glu Val Phe Ala Tyr Pro Ser Ala Thr Ile Ser Trp
            425   430   435

ttt cgg gat ggc cag ctg ctg cca agc tcc aat tac agc aat atc aag   1399
Phe Arg Asp Gly Gln Leu Leu Pro Ser Ser Asn Tyr Ser Asn Ile Lys
            440   445   450

atc tac aac acc ccc tct gcc agc tat ctg gag gtg acc cca gac tct   1447

Ile Tyr Asn Thr Pro Ser Ala Ser Tyr Leu Glu Val Thr Pro Asp Ser
455   460   465   470

gag aat gat ttt ggg aac tac aac tgt act gca gtg aac cgc att ggg   1495
Glu Asn Asp Phe Gly Asn Tyr Asn Cys Thr Ala Val Asn Arg Ile Gly
                   475   480   485

cag gag tcc ttc gaa ttc atc ctt gtt caa gca gac acc ccc tct tca   1543
Gln Glu Ser Phe Glu Phe Ile Leu Val Gln Ala Asp Thr Pro Ser Ser
              490   495   500

cca tcc atc gac cag gtg gag cca tac tcc agc aca gcc cag gtg cag   1591
Pro Ser Ile Asp Gln Val Glu Pro Tyr Ser Ser Thr Ala Gln Val Gln
         505   510   515

ttt gat gaa cca gag gcc aca ggt gag gtg ccc atc ctc aaa tac aaa , ·1639
Phe Asp Glu Pro Glu Ala Thr Gly Gly Val Pro Ile Leu Lys Tyr Lys
         520   525   530

gct gag tgg aga gca gtg ggt gaa gaa gta tgg cat tcc aag tgg tat   1687
Ala Glu Trp Arg Ala Val Gly Glu Glu Val Trp His Ser Lys Trp Tyr
535   540   545   550

gat gcc aag gaa gcc agc atg gag ggc atc gtc acc atc gtg ggc ctg   1735
Asp Ala Lys Glu Ala Ser Met Glu Gly Ile Val Thr Ile Val Gly Leu
              555   560   565

aag ccc gaa aca acg tac gcc gta agg ctg gcg gcg ctc aat ggc aaa   1783
Lys Pro Glu Thr Thr Tyr Ala Val Arg Leu Ala Ala Leu Asn Gly Lys
              570   575   580

ggg ctg ggt gag atc agc gcg gcc tcc gag ttc aag acg cag cca gtc   1831
Gly Leu Gly Glu Ile Ser Ala Ala Ser Glu Phe Lys Thr Gln Pro Val
         585   590   595

caa ggg gaa ccc agt gca cct aag ctc gaa ggg cag atg gga gag gat   1879

Gln Gly Glu Pro Ser Ala Pro Lys Leu Glu Gly Gln Met Gly Glu Asp
       600   605   610

gga aac tct att aaa gtg aac ctg atc aag cag gat gac gac ggc tcc   1927
Gly Asn Ser Ile Lys Val Asn Leu Ile Lys Gln Asp Asp Gly Gly Ser
615   620   625   630

ccc atc agg cac tat ctg gtc agg tac cga gcg ctc tcc tcc gag tgg   1975
Pro Ile Arg His Tyr Leu Val Arg Tyr Arg Ala Leu Ser Ser Glu Trp
               635   640   645

aaa cca gag atc agg ctc ccg tct ggc agt gac cac gtc atg ctg aag   2023
Lys Pro Glu Ile Arg Leu Pro Ser Gly Ser Asp His Val Met Leu Lys
           650   655   660

tcc ctg gac tgg aat gct gag tat gag gtc tac gtg gtg gct gag aac   2071
Ser Leu Asp Trp Asn Ala Glu Tyr Glu Val Tyr Val Val Ala Glu Asn
       665   670   675

cag caa gga aaa tcc aag gcg gct cat ttt gtg ttc agg acc tcg gcc   2119
Gln Gln Gly Lys Ser Lys Ala Ala His Phe Val Phe Arg Thr Ser Ala
   680   685   690

cag ccc aca gcc atc cca gcc aac ggc agc ccc acc tca ggc ctg agc   2167
Gln Pro Thr Ala Ile Pro Ala Asn Gly Ser Pro Thr Ser Gly Leu Ser
695   700   705   710

acc ggg gcc atc gtg ggc atc ctc atc gtc atc ttc gtc ctc ctg   2215
Thr Gly Ala Ile Val Gly Ile Leu Ile Val Ile Phe Val Leu Leu Leu
               715   720   725

gtg gtt gtg gac atc acc tgc tac ttc ctg aac aag tgt ggc ctg ttc   2263
Val Val Val Asp Ile Thr Cys Tyr Phe Leu Asn Lys Cys Gly Leu Phe
           730   735   740

atg tgc att gcg gtc aac ctg tgt gga aaa gcc ggg ccc ggg gcc aag   2311

Met Cys Ile Ala Val Asp Leu Cys Gly Lys Ala Gly Pro Gly Ala Lys
          745   750   755

ggc aag gac atg gag gag ggc aag gcc gcc ttc tcg aaa gat gag tcc   2359
Gly Lys Asp Met Glu Glu Gly Lys Ala Ala Phe Ser Lys Asp Glu Ser
          760   765   770

aag gag ccc atc gtg gag gtt aga acg gag gag gag agg acc cca aac   2407
Lys Glu Pro Ile Val Glu Val Arg Thr Glu Glu Glu Arg Thr Pro Asn
775   780   785   790

cat gat gga ggg aaa cac aca gag ccc aac gag acc acg cca ctg acg   2455
His Asp Gly Gly Lys His Thr Glu Pro Asn Glu Thr Thr Pro Leu Thr
                    795   800   805

gag ccc gag aag ggc ccc gta gaa aca aag cca gag tgc cag gag aca   2503
Glu Pro Glu Lys Gly Pro Val Glu Ala Lys Pro Glu Cys Gln Glu Thr
                    810   815   820

gaa acg aag cca gcg cca gcc gaa gtc aag acg gtc ccc aat gac gcc   2551
Glu Thr Lys Pro Ala Pro Ala Glu Val Lys Thr Val Pro Asn Asp Ala
          825   830   835

aca cag aca aag gag aac gag agc aaa gca tga tggggtgaaga gaaccgagca   2604
Thr Gln Thr Lys Glu Asn Glu Ser Lys Ala
          840   845

aagatcaaaa taaaaagtga cacagcagc   2633

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Ala Val Ser Leu Gln Val Asp Ile Val Pro Ser Gln Gly Glu Ile Ser
            20   25   30

Val Gly Glu Ser Lys Phe Phe Leu Cys Gln Val Ala Gly Asp Ala Lys
            35   40   45

Asp Lys Asp Ile Ser Trp Phe Ser Pro Asn Gly Glu Lys Leu Thr Pro
        50   55   60

Asn Gln Gln Arg Ile Ser Val Val Trp Asn Asp Asp Ser Ser Ser Thr
65   70   75   80

Leu Thr Ile Tyr Asn Ala Asn Ile Asp Asp Ala Gly Ile Tyr Lys Cys
                85   90   95

Val Val Thr Gly Glu Asp Gly Ser Glu Ser Glu Ala Thr Val Asn Val
                100   105   110

Lys Ile Phe Gln Lys Leu Met Phe Lys Asn Ala Pro Thr Pro Gln Glu
            115   120   125

Phe Arg Glu Gly Glu Asp Ala Val Ile Val Cys Asp Val Val Ser Ser
        130   135   140

Leu Pro Pro Thr Ile Ile Trp Lys His Lys Gly Arg Asp Val Ile Leu
145   150   155   160

Lys Lys Asp Val Arg Phe Ile Val Leu Ser Asn Asn Tyr Leu Gln Ile
                165   170   175

Arg Gly Ile Lys Lys Thr Asp Glu Gly Thr Tyr Arg Cys Glu Gly Arg
                180   185   190

Ile Leu Ala Arg Gly Glu Ile Asn Phe Lys Asp Ile Gln Val Ile Val
                195   200   205

Asn Val Pro Pro Thr Ile Arg Ala Arg Gln Asn Ile Val Asn Ala Thr
    210   215   220

Ala Asn Leu Gly Gln Ser Val Thr Leu Val Cys Asp Ala Glu Arg Phe
225   230   235   240

Pro Glu Pro Thr Met Ser Trp Thr Lys Asp Gly Glu Gln Ile Glu Gln
                245   250   255

Glu Glu Asp Asp Glu Lys Tyr Ile Phe Ser Asp Asp Ser Ser Gln Leu
                260   265   270

Thr Ile Lys Lys Val Asp Lys Asn Asp Glu Ala Glu Tyr Ile Cys Ile
        275   280   285

Ala Glu Asn Lys Ala Gly Glu Gln Asp Ala Thr Ile His Leu Lys Val
        290   295   300

Phe Ala Lys Pro Lys Ile Thr Tyr Val Glu Asn Gln Thr Ala Met Glu
305   310   315   320

Leu Glu Glu Gln Val Thr Leu Thr Cys Glu Ala Ser Gly Asp Pro Ile
                  325   330   335

Pro Ser Ile Thr Trp Arg Thr Ser Thr Arg Asn Ile Ser Ser Glu Glu
                  340   345   350

Lys Thr Leu Asp Gly His Met Val Val Arg Ser His Ala Arg Val Ser
        355   360   365

Ser Leu Thr Leu Lys Ser Ile Gln Tyr Thr Asp Ala Gly Glu Tyr Ile
        370   375   380

Cys Thr Ala Ser Asn Thr Ile Gly Gln Asp Ser Gln Ser Met Tyr Leu
385   390   395   400

Glu Val Gln Tyr Ala Pro Lys Leu Gln Gly Pro Val Ala Val Tyr Thr
                  405   410   415

Trp Glu Gly Asn Gln Val Asn Ile Thr Cys Glu Val Phe Ala Tyr Pro
                  420   425   430

Ser Ala Thr Ile Ser Trp Phe Arg Asp Gly Gln Leu Leu Pro Ser Ser
        435   440   445

Asn Tyr Ser Asn Ile Lys Ile Tyr Asn Thr Pro Ser Ala Ser Tyr Leu
    450   455   460

Glu Val Thr Pro Asp Ser Glu Asn Asp Phe Gly Asn Tyr Asn Cys Thr
465   470   475   480

Ala Val Asn Arg Ile Gly Gln Glu Ser Phe Glu Phe Ile Leu Val Gln
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Ala Asp Thr Pro Ser Ser Pro Ser Ile Asp Gln Val Glu Pro Tyr Ser
            500   505   510

Ser Thr Ala Gln Val Gln Phe Asp Glu Pro Glu Ala Thr Gly Gly Val
        515   520   525

Pro Ile Leu Lys Tyr Lys Ala Glu Trp Arg Ala Val Gly Glu Glu Val
    530   535   540

Trp His Ser Lys Trp Tyr Asp Ala Lys Glu Ala Ser Met Glu Gly Ile
545   550   555   560

Val Thr Ile Val Gly Leu Lys Pro Glu Thr Thr Tyr Ala Val Arg Leu
                565   570   575

Ala Ala Leu Asn Gly Lys Gly Leu Gly Glu Ile Ser Ala Ala Ser Glu
       580   585   590

Phe Lys Thr Gln Pro Val Gln Gly Glu Pro Ser Ala Pro Lys Leu Glu
     595   600   605

Gly Gln Met Gly Glu Asp Gly Asn Ser Ile Lys Val Asn Leu Ile Lys
   610   615   620

Gln Asp Asp Gly Gly Ser Pro Ile Arg His Tyr Leu Val Arg Tyr Arg
625   630   635   640

Ala Leu Ser Ser Glu Trp Lys Pro Glu Ile Arg Leu Pro Ser Gly Ser
       645   650   655

Asp His Val Met Leu Lys Ser Leu Asp Trp Asn Ala Glu Tyr Glu Val
       660   665   670

Tyr Val Val Ala Glu Asn Gln Gln Gly Lys Ser Lys Ala Ala His Phe
     675   680   685

Val Phe Arg Thr Ser Ala Gln Pro Thr Ala Ile Pro Ala Asn Gly Ser
     690   695   700

Pro Thr Ser Gly Leu Ser Thr Gly Ala Ile Val Gly Ile Leu Ile Val
705   710   715   720

Ile Phe Val Leu Leu Leu Val Val Val Asp Ile Thr Cys Tyr Phe Leu
               725   730   735

Asn Lys Cys Gly Leu Phe Met Cys Ile Ala Val Asn Leu Cys Gly Lys
          740   745   750

Ala Gly Pro Gly Ala Lys Gly Lys Asp Met Glu Glu Gly Lys Ala Ala
          755   760   765

Phe Ser Lys Asp Glu Ser Lys Glu Pro Ile Val Glu Val Arg Thr Glu
     770   775   780

Glu Glu Arg Thr Pro Asn His Asp Gly Gly Lys His Thr Glu Pro Asn
785   790   795   800

Glu Thr Thr Pro Leu Thr Glu Pro Glu Lys Gly Pro Val Glu Ala Lys
               805   810   815

Pro Glu Cys Gln Glu Thr Glu Thr Lys Pro Ala Pro Ala Glu Val Lys
          820   825   830

Thr Val Pro Asn Asp Ala Thr Gln Thr Lys Glu Asn Glu Ser Lys Ala
          835   840   845

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atg gag cta ctg tcg cca ccg ctc cgc gac gta gac ctg acg gcc ccc 168
Met Glu Leu Leu Ser Pro Pro Leu Arg Asp Val Asp Leu Thr Ala Pro
1   5   10   15

gac ggc tct ctc tgc tcc ttt gcc aca acg gac gac ttc tat gac gac 216
Asp Gly Ser Leu Cys Ser Phe Ala Thr Thr Asp Asp Phe Tyr Asp Asp
            20   25   30

ccg tgt ttc gac tcc ccg gac ctg cgc ttc ttc gaa gac ctg gac ccg 264
Pro Cys Phe Asp Ser Pro Asp Leu Arg Phe Phe Glu Asp Leu Asp Pro
        35   40   45

cgc ctg atg cac gtg ggc gcg ctc ctg aaa ccc gaa gag cac tcg cac 312
Arg Leu Met His Val Gly Ala Leu Leu Lys Pro Glu Glu His Ser His
    50   55   60

ttc ccc gcg gcg gtg cac ccg gcc ccg ggc gca cgt gag gac gag cat 360
Phe Pro Ala Ala Val His Pro Ala Pro Gly Ala Arg Glu Asp Glu His
65   70   75   80

gtg cgc gcg ccc agc ggg cac cac cag gcg ggc cgc tgc cta ctg tgg 408
Val Arg Ala Pro Ser Gly His His Gln Ala Gly Arg Cys Leu Leu Trp
                85   90   95

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gcc tgc aag gcg tgc aag cgc aag acc acc aac gcc gac agc cgc aag    456
Ala Cys Lys Ala Cys Lys Arg Lys Thr Thr Asn Ala Asp Arg Arg Lys
            100               105               110
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gcc gcc acc atg cgc gag cgg cgc cgc ctg agc aaa gta aat gag gcc    504
Ala Ala Thr Met Arg Glu Arg Arg Arg Leu Ser Lys Val Asn Glu Ala
            115               120               125
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```
ttt gag aca ctc aag cgc tgc acg tcg agc aat cca aac cag cgg ttg    552
Phe Glu Thr Leu Lys Arg Cys Thr Ser Ser Asn Pro Asn Gln Arg Leu
            130               135               140
```

```
ccc aag gtg gag atc ctg cgc aac gcc atc cgc tat atc gag ggc ctg    600
Pro Lys Val Glu Ile Leu Arg Asn Ala Ile Arg Tyr Ile Glu Gly Leu
145               150               155               160
```

```
cag gct ctg ctg cgc gac cag gac gcc gcg ccc cct ggc gca gcc gcc    648
Gln Ala Leu Leu Arg Asp Gln Asp Ala Ala Pro Pro Gly Ala Ala Ala
            165               170               175
```

```
ttc tat gcg ccg ggc ccg ctg ccc ccg ggc cgc ggc ggc gag cac tac    696
Phe Tyr Ala Pro Gly Pro Leu Pro Pro Gly Arg Gly Gly Glu His Tyr
            180               185               190
```

```
agc ggc gac tcc gac gcg tcc agc ccg cgc tcc aac tgc tcc gac ggc    744
Ser Gly Asp Ser Asp Ala Ser Ser Pro Arg Ser Asn Cys Ser Asp Gly
            195               200               205
```

```
atg atg gac tac agc ggc ccc ccg agc ggc gca cgg cgg cgg aac tgc    792
Met Met Asp Tyr Ser Gly Pro Pro Ser Gly Ala Arg Arg Arg Asn Cys
            210               215               220
```

```
tac gaa ggc gcc tac tac aac gag gcg ccc agc gaa ccc agg ccc gga    840
Tyr Glu Gly Ala Tyr Tyr Asn Glu Ala Pro Ser Glu Pro Arg Pro Gly
225               230               235               240
```

```
aag agt gcg gcg gtg tcg agc cta gac tac ctg tcc agc atc gtg gag      888
Lys Ser Ala Ala Val Ser Ser Leu Asp Tyr Leu Ser Ser Ile Val Glu
              245               250               255

cgc atc tcc acc gag agc cct gcg gcg ccc gcc ctc ctg ctg gcg gac      936
Arg Ile Ser Thr Glu Ser Pro Ala Ala Pro Ala Leu Leu Leu Ala Asp
              260               265               270

gtg cct tct gag tcg cct ccg cgc agg caa gag gct gcc gcc ccc agc      984
Val Pro Ser Glu Ser Pro Pro Arg Arg Gln Glu Ala Ala Ala Pro Ser
          275               280               285

gag gga gag agc agc ggc gac ccc acc cag tca ccg gac gcc gcc ccg     1032
Glu Gly Glu Ser Ser Gly Asp Pro Thr Gln Ser Pro Asp Ala Ala Pro
      290               295               300

cag tgc cct gcg ggt gcg aac ccc aac ccg ata tac cag gtg ctc tga     1080
Gln Cys Pro Ala Gly Ala Asn Pro Asn Pro Ile Tyr Gln Val Leu
306               310               315

gagggatgtg gcgccaac cccgccaggg atggtgccct agggtccctc gcgcccaaac     1140

gattgaactt aaatgccccc ctccaacag cgctttaaaa gcgccatctc ttgaggtagg     1200

agaggcggag aactguagtt tccgccccac ccgacagggc aaggacacag cgcggttttt     1260

tccacgcagc acccttctcg gagacccatt gcgatggccg ctccgtgttc ctcggtgggc     1320

cagagctgaa ccttgagggg ctaggttcac gtttctcgcg ccctccatgg tgagaccctc     1380

gcagccctaa ccctgccccg ggatgcaccg gttatttggg gaggcgtgag acagtgcact     1440

ccggtcccaa atgtagcagg tgtaaccgta accaccccc aacccgtttc ccggttcagg     1500

acccctttt gtaataccttt ttgtaatcta ttcctgtaaa taagagttcg tttgccagag     1560
```

aggagcccct gagggtgtat tttctctctga ggcagggtgt gtggtgctac agggaatttg   1620

tacgtttata ccgcaggcgg gcgagccgcg ggcgctcgct caggtgatca aaataaaggc   1680

gctaattttt aa   1692

<210> 10
<211> 319
<212> PRT
<213> Homo sapiens

<400> 10

Met Glu Leu Leu Ser Pro Pro Leu Arg Asp Val Asp Leu Thr Ala Pro
1   5   10   15

Asp Gly Ser Leu Cys Ser Phe Ala Thr Thr Asp Asp Phe Tyr Asp Asp
            20   25   30

Pro Cys Phe Asp Ser Pro Asp Leu Arg Phe Phe Glu Asp Leu Asp Pro
        35   40   45

Arg Leu Met His Val Gly Ala Leu Leu Lys Pro Glu Glu His Ser His
    50   55   60

Phe Pro Ala Ala Val His Pro Ala Pro Gly Ala Arg Glu Asp Glu His
65   70   75   80

Val Arg Ala Pro Ser Gly His His Gln Ala Gly Arg Cys Leu Leu Trp
            85   90   95

Ala Cys Lys Ala Cys Lys Arg Lys Thr Thr Asn Ala Asp Arg Arg Lys
            100   105   110

Ala Ala Thr Met Arg Glu Arg Arg Arg Leu Ser Lys Val Asn Glu Ala
        115   120   125

Phe Glu Thr Leu Lys Arg Cys Thr Ser Ser Asn Pro Asn Gln Arg Leu
    130   135   140

Pro Lys Val Glu Ile Leu Arg Asn Ala Ile Arg Tyr Ile Glu Gly Leu
145   150   155   160

Gln Ala Leu Leu Arg Asp Gln Asp Ala Ala Pro Pro Gly Ala Ala Ala
            165   170   175

Phe Tyr Ala Pro Gly Pro Leu Pro Pro Gly Arg Gly Gly Glu His Tyr
            180   185   190

Ser Gly Asp Ser Asp Ala Ser Ser Pro Arg Ser Asn Cys Ser Asp Gly
            195   200   205

Met Met Asp Tyr Ser Gly Pro Pro Ser Gly Ala Arg Arg Arg Asn Cys
    210   215   220

Tyr Glu Gly Ala Tyr Tyr Asn Glu Ala Pro Ser Glu Pro Arg Pro Gly
225   230   235   240

Lys Ser Ala Ala Val Ser Ser Leu Asp Tyr Leu Ser Ser Ile Val Glu
              245   250   255

Arg Ile Ser Thr Glu Ser Pro Ala Ala Pro Ala Leu Leu Leu Ala Asp
              260   265   270

Val Pro Ser Glu Ser Pro Pro Arg Arg Gln Glu Ala Ala Ala Pro Ser
              275   280   285

Glu Gly Glu Ser Ser Gly Asp Pro Thr Gln Ser Pro Asp Ala Ala Pro
       290   295   300

Gln Cys Pro Ala Gly Ala Asn Pro Asn Pro Ile Tyr Gln Val Leu
305   310   315

<210> 11
<211> 1427
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (43)..(810)

<400> 11
cctctcgctg ccgtccaggt gcaccgcctg cctctcagcc gg atg gac gtg atg   54
                                                 Met Asp Val Met
                                                  1

```
gat ggc tgc cag ttc tca cct tct gag tac ttc tac gac ggc tcc tgc      102
Asp Gly Cys Gln Phe Ser Pro Ser Glu Tyr Phe Tyr Asp Gly Ser Cys
5                   10                  15                  20

ata ccg tcc ccc gag ggt gaa ttt ggg gac gag ttt gtg ccg cga gtg      150
Ile Pro Ser Pro Glu Gly Glu Phe Gly Asp Glu Phe Val Pro Arg Val
                    25                  30                  35

gct gcc ttc gga gcg cac aaa gca gag ctg cag ggc tca gat gag gac      198
Ala Ala Phe Gly Ala His Lys Ala Glu Leu Gln Gly Ser Asp Glu Asp
                40                  45                  50

gag cac gtg cga gcg cct acc ggc cac cac cag gct ggt cac tgc ctc      246
Glu His Val Arg Ala Pro Thr Gly His His Gln Ala Gly His Cys Leu
            55                  60                  65

atg tgg gcc tgc aaa gcc tgc aag agg aag tcc acc acc atg gat cgg      294
Met Trp Ala Cys Lys Ala Cys Lys Arg Lys Ser Thr Thr Met Asp Arg
        70                  75                  80

cgg aag gcc gcc act atg cgc gag cgg agg cgc ctg aag aag gtc aac      342
Arg Lys Ala Ala Thr Met Arg Glu Arg Arg Arg Leu Lys Lys Val Asn
85                  90                  95                  100

cag gct ttc gaa acc ctc aag agg tgt acc acg acc aac ccc aac cag      390
Gln Ala Phe Glu Thr Leu Lys Arg Cys Thr Thr Thr Asn Pro Asn Gln
                105                 110                 115

agg ctg ccc aag gtg gag atc ctc agg aat gcc atc cgc tac atc gag      438
Arg Leu Pro Lys Val Glu Ile Leu Arg Asn Ala Ile Arg Tyr Ile Glu
                120                 125                 130

agc ctg cag gag ttg ctg aga gag cag gtg gag aac tac tat agc ctg      486
Ser Leu Gln Glu Leu Leu Arg Glu Gln Val Glu Asn Tyr Tyr Ser Leu
        135                 140                 145
```

ccg gga cag agc tgc tcg gag ccc acc agc ccc acc tcc aac tgc tct   534
Pro Gly Gln Ser Cys Ser Glu Pro Thr Ser Pro Thr Ser Asn Cys Ser
     150   155   160

gat ggc atg ccc gaa tgt aac agt cct gtc tgg tcc aga aag agc agt   582
Asp Gly Met Pro Glu Cys Asn Ser Pro Val Trp Ser Arg Lys Ser Ser
165   170   175   180

act ttt gac agc atc tac tgt cct gat gta tca aat gta tat gcc aca   630
Thr Phe Asp Ser Ile Tyr Cys Pro Asp Val Ser Asn Val Tyr Ala Thr
                 185   190   195

gat aaa aac tcc tta tcc agc ttg gat tgc tta tcc aac ata gtg gac   678
Asp Lys Asn Ser Leu Ser Ser Leu Asp Cys Leu Ser Asn Ile Val Asp
             200   205   210

cgg atc acc tcc tca gag caa cct ggg ttg cct ctc cag gat ctg gct   726
Arg Ile Thr Ser Ser Glu Gln Pro Gly Leu Pro Leu Gln Asp Leu Ala
         215   220   225

tat ctc tct cca gtt gcc agc acc gat tca cag cct cga act cca ggg   774
Ser Leu Ser Pro Val Ala Ser Thr Asp Ser Gln Pro Arg Thr Pro Gly
     230   235   240

gct tct agt tcc agg ctt atc tat cat gtg cta tga actaatttc   820
Ala Ser Ser Ser Arg Leu Ile Tyr His Val Leu
245   250   255

tggtctatat gacttcttcc aggagggcct aatacacagg acgaagaagg cttcaaaaag   880

tcccaaccca agacaacatg tacatacatga tttctttca gttgtaaatt tgtaaagatt   940

acettgccac tttataagaa agtgtattta actaaaagt catcattgca aataatactt   1000

tcttcttctt tattattctt tgcttagata ttaataacata gttccagtaa tactatttct   1060

gataggggge cattgattga gggtagcttg ttcgaatgct taacttatat atacatatat   1120

atatattata aatattgctc atcaaaatgt ctctggtgtt tagagcttta ttttttcctt   1180

taaaacatta aaacagctga gaatcagtta aatggaattt taaatatatt taactattto   1240

tttctotctt aatcctttag ttatattgta ttaaatataa atataatact gcctaatgta   1300

tatattttga tattttcttg taagaaatgt atcttttaaa tgtaagcaca aaatagtact   1360

ttgtggatca tttcaagata taagaaattt tggaaattcc accataaata aaatttttta   1420

ctacaag   1427

<210> 12
<211> 255
<212> PRT
<213> Homo sapiens

<400> 12

Met Asp Val Met Asp Gly Cys Gln Phe Ser Pro Ser Glu Tyr Phe Tyr
1   5   10   15

Asp Gly Ser Cys Ile Pro Ser Pro Glu Gly Glu Phe Gly Asp Glu Phe
            20   25   30

Val Pro Arg Val Ala Ala Phe Gly Ala His Lys Ala Glu Leu Gln Gly
        35   40   45

Ser Asp Glu Asp Glu His Val Arg Ala Pro Thr Gly His His Gln Ala

50   55   60

Gly His Cys Leu Met Trp Ala Cys Lys Ala Cys Lys Arg Lys Ser Thr
65   70   75   80

Thr Met Asp Arg Arg Lys Ala Ala Thr Met Arg Glu Arg Arg Arg Leu
                  85   90   95

Lys Lys Val Asn Gln Ala Phe Glu Thr Leu Lys Arg Cys Thr Thr Thr
              100   105   110

Asn Pro Asn Gln Arg Leu Pro Lys Val Glu Ile Leu Arg Asn Ala Ile
          115   120   125

Arg Tyr Ile Glu Ser Leu Gln Glu Leu Leu Arg Glu Gln Val Glu Asn
      130   135   140

Tyr Tyr Ser Leu Pro Gly Gln Ser Cys Ser Glu Pro Thr Ser Pro Thr
146   150   155   160

Ser Asn Cys Ser Asp Gly Met Pro Glu Cys Asn Ser Pro Val Trp Ser
              165   170   175

Arg Lys Ser Ser Thr Phe Asp Ser Ile Tyr Cys Pro Asp Val Ser Asn
              180   185   190

Val Tyr Ala Thr Asp Lys Asn Ser Leu Ser Ser Leu Asp Cys Leu Ser

```
              195                  200                  205
```

Asn Ile Val Asp Arg Ile Thr Ser Ser Glu Gln Pro Gly Leu Pro Leu
```
     210                  215                  220
```

Gln Asp Leu Ala Ser Leu Ser Pro Val Ala Ser Thr Asp Ser Gln Pro
```
225                  230                  235                  240
```

Arg Thr Pro Gly Ala Ser Ser Ser Arg Leu Ile Tyr His Val Leu
```
                245                  250                  255
```

<210> 13
<211> 675
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(675)

<400> 13
atg gag ctg tat gag aca tcc ccc tac ttc tac cag gag ccc cgc ttc   48
Met Glu Leu Tyr Glu Thr Ser Pro Tyr Phe Tyr Gln Glu Pro Arg Phe
1   5   10   15

tat gat ggg gag aac tac ctg cct gtc cac ctc cag ggc ttc gag cca   96
Tyr Asp Gly Glu Asn Tyr Leu Pro Val His Leu Gln Gly Phe Glu Pro
            20   25   30

cca ggc tac gag cgg acg gag ctc acc ctg agc ccc gag gcc cca ggg   144
Pro Gly Tyr Glu Arg Thr Glu Leu Thr Leu Ser Pro Glu Ala Pro Gly

```
              35                    40                    45

ccc ctt gag gac aag ggg ctg ggg acc ccc gag cac tgt cca ggc cag    192
Pro Leu Glu Asp Lys Gly Leu Gly Thr Pro Glu His Cys Pro Gly Gln
    50                    55                    60

tgc ctg ccg tgg gcg tgt aag gtg tgt aag agg aag tcg gtg tcc gtg    240
Cys Leu Pro Trp Ala Cys Lys Val Cys Lys Arg Lys Ser Val Ser Val
55                    70                    75                    80

gac cgg cgg cgg gcg gcc aca ctg agg gag aag cgc agg ctc aag aag    288
Asp Arg Arg Arg Ala Ala Thr Leu Arg Glu Lys Arg Arg Leu Lys Lys
                  85                    90                    95

gtg aat gag gcc ttc gag gcc ctg aag aga agc acc ctg ctc aac ccc    336
Val Asn Glu Ala Phe Glu Ala Leu Lys Arg Ser Thr Leu Leu Asn Pro
                  100                   105                   110

aac cag cgg ctg ccc aag gtg gag atc ctg cgc agt gcc atc cag tac    384
Asn Gln Arg Leu Pro Lys Val Glu Ile Leu Arg Ser Ala Ile Gln Tyr
                  115                   120                   125

atc gag cgc ctc cag gcc ctg ctc agc tcc ctc aac cag gag gag cgt    432
Ile Glu Arg Leu Gln Ala Leu Leu Ser Ser Leu Asn Gln Glu Glu Arg
      130                   135                   140

gac ctc cgc tac cgg ggc ggg ggc ggg ccc cag cca ggg gtg ccc agc    480
Asp Leu Arg Tyr Arg Gly Gly Gly Gly Pro Gln Pro Gly Val Pro Ser
145                   150                   155                   160

gaa tgc agc tct cac agc gcc tcc tgc agt cca gag tgg ggc agt gca    528
Glu Cys Ser Ser His Ser Ala Ser Cys Ser Pro Glu Trp Gly Ser Ala
                  165                   170                   175

ctg gag ttc agc gcc aac cca ggg gat cat ctg ctc acg gct gac cct    576
Leu Glu Phe Ser Ala Asn Pro Gly Asp His Leu Leu Thr Ala Asp Pro
```

```
                180              185              190

aca gat gcc cac aac ctg cac tcc ctc acc tcc atc gtg gac agc atc    624
Thr Asp Ala His Asn Leu His Ser Leu Thr Ser Ile Val Asp Ser Ile
         195              200              205

aca gtg gaa gat gtg tct gtg gcc ttc cca gat gaa acc atg ccc aac    672
Thr Val Glu Asp Val Ser Val Ala Phe Pro Asp Glu Thr Met Pro Asn
    210              215              220

tag                                                                675
```

<210> 14
<211> 224
<212> PRT
<213> Homo sapiens

<400> 14

```
Met Glu Leu Tyr Glu Thr Ser Pro Tyr Phe Tyr Gln Glu Pro Arg Phe
1                5                10               15


Tyr Asp Gly Glu Asn Tyr Leu Pro Val His Leu Gln Gly Phe Glu Pro
            20               25               30


Pro Gly Tyr Glu Arg Thr Glu Leu Thr Leu Ser Pro Glu Ala Pro Gly
        35               40               45


Pro Leu Glu Asp Lys Gly Leu Gly Thr Pro Glu His Cys Pro Gly Gln
    50               55               60
```

Cys Leu Pro Trp Ala Cys Lys Val Cys Lys Arg Lys Ser Val Ser Val
65   70   75   80

Asp Arg Arg Arg Ala Ala Thr Leu Arg Glu Lys Arg Arg Leu Lys Lys
                   85   90   95

Val Asn Glu Ala Phe Glu Ala Leu Lys Arg Ser Thr Leu Leu Asn Pro
                   100   105   110

Asn Gln Arg Leu Pro Lys Val Glu Ile Leu Arg Ser Ala Ile Gln Tyr
          115   120   125

Ile Glu Arg Leu Gln Ala Leu Leu Ser Ser Leu Asn Gln Glu Glu Arg
     130   135   140

Asp Leu Arg Tyr Arg Gly Gly Gly Gly Pro Gln Pro Gly Val Pro Ser
145   150   155   160

Glu Cys Ser Ser His Ser Ala Ser Cys Ser Pro Glu Trp Gly Ser Ala
                   165   170   175

Leu Glu Phe Ser Ala Asn Pro Gly Asp His Leu Leu Thr Ala Asp Pro
          180   185   190

Thr Asp Ala His Asn Leu His Ser Leu Thr Ser Ile Val Asp Ser Ile
          195   200   205

Thr Val Glu Asp Val Ser Val Ala Phe Pro Asp Glu Thr Met Pro Asn
         210   215   220

```
<210>  15
<211>  3935
<212>  DNA
<213>  Homo sapiens


<220>
<221>  CDS
<222>  (373)..(1902)


<400>  15
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ggagagccga aagcggagct cgaaactgac tggaaacttc agtggcgcgg agactcgcca   60

gtttcaaccc cggaaacttt tctttgcacg aggagaagag aagggtgca agcgcccccc   120

cttttgctct tttcctccc ctcctcctcc tctccaattc gcctcccccc acttggagcg   180

agcagctgtg aactggccac cccgcgcctt cctaagtgct cgccgcggta gccggccgac   240

gcgccagctt ccccggcagc cgcttgctcc gcatccggc agccgaggg agaggagccc   300

gcgcctcgag tccccgagcc gccgccgctt ctcgcctttc ccggccacca gccccctgcc   360

ccggcccccgc gt atg aat ctc ctg gac ccc ttc atg aag atg acc gac gag   411
               Met Asn Leu Leu Asp Pro Phe Met Lys Met Thr Asp Glu
               1   5   10

cag gag aag ggc ctg tcc ggc gcc ccc agc ccc acc atg tcc gag gac   459
Gln Glu Lys Gly Leu Ser Gly Ala Pro Ser Pro Thr Met Ser Glu Asp
15   20   25

tcc gcg ggc tcg ccc tgc cag tcg ggc tcc ggc tcg gac acc gag aac   507

```
                Ser Ala Gly Ser Pro Cys Pro Ser Gly Ser Gly Ser Asp Thr Glu Asn
                30                  35                  40                  45

acg cgg ccc cag gag aac acg ttc ccc aag ggc gag ccc gat ctg aag        555
Thr Arg Pro Gln Glu Asn Thr Phe Pro Lys Gly Glu Pro Asp Leu Lys
                    50                  55                  60

aag gag agc gag gag gac aag ttc ccc gtg tgc atc cgc gag gcg gtc        603
Lys Glu Ser Glu Glu Asp Lys Phe Pro Val Cys Ile Arg Glu Ala Val
                65                  70                  75

agc cag gtg ctc aaa ggc tac gac tgg acg ctg gtg ccc atg ccg gtg        651
Ser Gln Val Leu Lys Gly Tyr Asp Trp Thr Leu Val Pro Met Pro Val
                80                  85                  90

cgc gtc aac ggc tcc agc aag aac aag ccg cac gtc aag cgg ccc atg        699
Arg Val Asn Gly Ser Ser Lys Asn Lys Pro His Val Lys Arg Pro Met
                95                  100                 105

aac gcc ttc atg gtg tgg gcg cag gcg gcg cgc agg aag ctc gcg gac        747
Asn Ala Phe Met Val Trp Ala Gln Ala Ala Arg Arg Lys Leu Ala Asp
110                 115                 120                 125

cag tac ccg cac ttg cac aac gcc gag ctc agc aag acg ctg ggc aag        795
Gln Tyr Pro His Leu His Asn Ala Glu Leu Ser Lys Thr Leu Gly Lys
                    130                 135                 140

ctc tgg aga ctt ctg aac gag agc gag aag cgg ccc ttc gtg gag gag        843
Leu Trp Arg Leu Leu Asn Glu Ser Glu Lys Arg Pro Phe Val Glu Glu
                145                 150                 155

gcg gag cgg ctg cgc gtg cag cac aag aag gac cac ccg gat tac aag        891
Ala Glu Arg Leu Arg Val Gln His Lys Lys Asp His Pro Asp Tyr Lys
                160                 165                 170

tac cag ccg cgg cgg agg aag tcg gtg aag aac ggg gcg gag gca        939
```

Tyr Gln Pro Arg Arg Arg Lys Ser Val Lys Asn Gly Gln Ala Glu Ala
      175   180   185

gag gag gcc acg gag cag acg cac atc tcc ccc aac gcc atc ttc aag   987
Glu Glu Ala Thr Glu Gln Thr His Ile Ser Pro Asn Ala Ile Phe Lys
190   195   200   205

gcg ctg cag gcc gac tcg cca cac tcc tcc tcc ggc atg agc gag gtg   1035
Ala Leu Gln Ala Asp Ser Pro His Ser Ser Ser Gly Met Ser Glu Val
                  210   215   220

cac tcc ccc ggc gag cac tcg ggg caa tcc cag ggc cca ccg acc cca   1083
His Ser Pro Gly Glu His Ser Gly Gln Ser Gln Gly Pro Pro Thr Pro
                  225   230   235

ccc acc acc ccc aag acc gac gtg cag ccg ggc aag gct gac ctg aag   1131
Pro Thr Thr Pro Lys Thr Asp Val Gln Pro Gly Lys Ala Asp Leu Lys
            240   245   250

cga gag ggg cgc ccc ttg cca gag ggg gga aga cag ccc cct atc gac   1179
Arg Glu Gly Arg Pro Leu Pro Glu Gly Gly Arg Gln Pro Pro Ile Asp
      255   260   265

ttc cgc gac gtg gac atc ggc gag ctg agc agc gac gtc atc tcc aac   1227
Phe Arg Asp Val Asp Ile Gly Glu Leu Ser Ser Asp Val Ile Ser Asn
270   275   280   285

atc gag acc ttc gat gtg aac gag ttt gac cag tac ctg ccg ccc aac   1275
Ile Glu Thr Phe Asp Val Asn Glu Phe Asp Gln Tyr Leu Pro Pro Asn
                  290   295   300

ggc cac ccg ggg gtg ccg gcc acg cac ggc cag gtc acc tac acg ggc   1323
Gly His Pro Gly Val Pro Ala Thr His Gly Gln Val Thr Tyr Thr Gly
            305   310   315

agc tac ggc atc agc agc acc gcg gcc acc ccg gcg agc gcg ggc cac   1371

Ser Tyr Gly Ile Ser Ser Thr Ala Ala Thr Pro Ala Ser Ala Gly His
         320   325   330

gtg tgg atg tcc aag cag cag gcg acg acg cca ccc ccg cag cag ccc   1419
Val Trp Met Ser Lys Gln Gln Ala Pro Pro Pro Pro Gln Gln Pro
     335   340   345

cca cag gcc ccg ccg gcc ccg cag gcg ccc ccg cag ccg cag gcg gcg   1467
Pro Gln Ala Pro Pro Ala Pro Gln Ala Pro Pro Gln Pro Gln Ala Ala
350   355   360   365

ccc cca cag cag ccg gcg gca ccc ccg cag cag cca cag gcg cac acg   1515
Pro Pro Gln Gln Pro Ala Ala Pro Pro Gln Gln Pro Gln Ala His Thr
                 370   375   380

ctg acc acg ctg agc agc gag ccg ggc cag tcc cag cga acg cac atc   1563
Leu Thr Thr Leu Ser Ser Glu Pro Gly Gln Ser Gln Arg Thr His Ile
                 385   390   395

aag acg gag cag ctg agc ccc agc cac tac agc gag cag cag cag cac   1611
Lys Thr Glu Gln Leu Ser Pro Ser His Tyr Ser Glu Gln Gln Gln His
         400   405   410

tcg ccc cas cag atc gcc tac agc ccc ttc aac ctc cca cac tac agc   1659
Ser Pro Gln Gln Ile Ala Tyr Ser Pro Phe Asn Leu Pro His Tyr Ser
     415   420   425

ccc tcc tac ccg ccc atc acc cgc tca cag tac gac tac acc gac cac   1707
Pro Ser Tyr Pro Pro Ile Thr Arg Ser Gln Tyr Asp Tyr Thr Asp His
430   435   440   445

cag aac tcc agc tcc tac tac agc cac gcg gca ggc cag ggc acc ggc   1755
Gln Asn Ser Ser Ser Tyr Tyr Ser His Ala Ala Gly Gln Gly Thr Gly
                 450   455   460

ctc tac tcc acc ttc acc tac atg aac ccc gct cag cgc ccc atg tac   1803

Leu Tyr Ser Thr Phe Thr Tyr Met Asn Pro Ala Gln Arg Pro Met Tyr
            465   470   475

acc ccc atc gcc gac acc tct gag gtc cct tcc atc ccg cag acc cac   1851
Thr Pro Ile Ala Asp Thr Ser Gly Val Pro Ser Ile Pro Gln Thr His
            480   485   490

agc ccc cag cac tgg gaa caa ccc gtc tac aca cag ctc act cga cct   1899
Ser Pro Gln His Trp Glu Gln Pro Val Tyr Thr Gln Leu Thr Arg Pro
        495   500   505

tga ggaggcctcc cacgaaggge gaagatggcc gagatgatcc taaaaataac   1952

cgaagaaaga gaggaccaac cagaattccc tttggacatt tgtgtttttt tgttttttta   2012

ttttgttttg ttttttcttc ttcttcttct tccttaaaga catttaagct aaaggcaact, 2072

cgtacccaaa tttccaagac acaaacatca cctatccaag cgcattaccc acttgttggcc   2132

aatcagtggc caggccaacc ttggctaaat gaagcagcga aatcaacgag aaactggact   2192

tttaaaccc tcttcagagc aagcgtggag gatgatggag aatcgtgtga tcagtgtgct   2252

aaatctctct goctgtttgg actttgtaat tattttttta gcagtaatta aagaaaaag   2312

tcctctgtga ggaatattct ctattttaaa tatttttagt atgtactgtg tatgattcat   2372

taccattttg aggggattta tacatatttt tagataaaat taaatgctct tatttttcca   2432

acagctaaac tactcttagt tgaacagtgt gccctagctt ttcttgcaac cagagtatt   2492

ttgtacagat ttgcttttct ttacaaaaag aaaaaaaaaa tcctgttgta ttaacattta   2552

aaaacagaat tgtgttatgt gatcagtttt gggggttaac tttgcttaat tcctcaggct   2612

ttgcgattta aggaggagct gccttaaaaa aaaataaagg cottattttg caattatggg   2672

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agtaaacaat agtotagaga agoatttggt aagcttate atatatatat tttttaaaga   2732

agagaaaaac aoottgagoo ttaaaacggt gotgotggga aacatttgca ctottttagt   2792

gcatttocte ctgcottgo ttgttoactg cagtottaag aaagaggtaa aaggcaagca   2852

aaggagatga aatctgttct gggaatgttt cagcagccaa taagtgcocg agcacactgc   2912

ccoaggttge ctgcotggge cocatgtgga aggcagatgc ctgcctgcctc tgtcacctgt   2972

goctotcaga acaccagoag ttaacottoa agacattcoa cttgctaaaa ttatttattt   3032

tgtaaggaga ggttttaatt aaaacaaaaa aaattcttt tttttttttt tttccaattt   3092

taccttcttt aaaataggtt gttggagott tcctcaaagg gtatggtcat ctgttgttaa  .3152

attatgttct taactgtaac cagttttttt ttatttatct ctttaatctt tttttattat   3212

taaaagcaag tttotttgta ttcctcacca tagatttgta taaatgcctt tttgtccatc   3272

cctttttct ttgttgtttt tgttgaaaao aaactgagaa cttgttctt tttttgtata   3332

aatgagagat tgcaaatgta gtgtatcact gagtcatttg cagtgtttc tgccacagac   3392

ctttgggctg cottatattg tgtgtgtgtg tgggtgtgtg tgtgttttga cacaaaaaca   3452

atgcaagcat gtgtcatcca tatttotcta catcttotct tggagtgagg gaggctacct   3512

ggaagggatc agcccactga cagacottaa tcttaattac tgctgtggot agagagtttg   3572

aggattgctt tttaaaaag acagcaaact tttttttta tttaaaaaaa gatatattas   3632

cagtttttaga agtcagtaga ataaaatctt aaagcactaa taatatggca tocttcaatt   3692

tctgtataaa agcagatctt tttaaaaga tacttotgta acttaagaaa cotggcattt   3752
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aastcatatt ttgtatttag gtaaaagctt tggtttgtgt tagtgttttg tttgtttcac   3812

ttgtttccct ccaagccccca aacctttgt tctctccgtg aaacttacct ttcccttttt   3872

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aaa   3935

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<400> 16

Met Asn Leu Leu Asp Pro Phe Met Lys Met Thr Asp Glu Gln Glu Lys
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Gly Leu Ser Gly Ala Pro Ser Pro Thr Met Ser Glu Asp Ser Ala Gly
            20   25   30

Ser Pro Cys Pro Ser Gly Ser Gly Ser Asp Thr Glu Asn Thr Arg Pro
        35   40   45

Gln Glu Asn Thr Phe Pro Lys Gly Glu Pro Asp Leu Lys Lys Glu Ser
    50   55   60

Glu Glu Asp Lys Phe Pro Val Cys Ile Arg Glu Ala Val Ser Gln Val
65   70   75   80

Leu Lys Gly Tyr Asp Trp Thr Leu Val Pro Met Pro Val Arg Val Asn
            85   90   95

Gly Ser Ser Lys Asn Lys Pro His Val Lys Arg Pro Met Asn Ala Phe
            100   105   110

Met Val Trp Ala Gln Ala Ala Arg Arg Lys Leu Ala Asp Gln Tyr Pro
        115   120   125

His Leu His Asn Ala Glu Leu Ser Lys Thr Leu Gly Lys Leu Trp Arg
    130   135   140

Leu Leu Asn Glu Ser Glu Lys Arg Pro Phe Val Glu Glu Ala Glu Arg
145   150   155   160

Leu Arg Val Gln His Lys Lys Asp His Pro Asp Tyr Lys Tyr Gln Pro
            165   170   175

Arg Arg Arg Lys Ser Val Lys Asn Gly Gln Ala Glu Ala Glu Glu Ala
            180   185   190

Thr Glu Gln Thr His Ile Ser Pro Asn Ala Ile Phe Lys Ala Leu Gln
        195   200   205

Ala Asp Ser Pro His Ser Ser Ser Gly Met Ser Glu Val His Ser Pro
    210   215   220

Gly Glu His Ser Gly Gln Ser Gln Gly Pro Pro Thr Pro Pro Thr Thr
225   230   235   240

Pro Lys Thr Asp Val Gln Pro Gly Lys Ala Asp Leu Lys Arg Glu Gly
                 245   250   255

Arg Pro Leu Pro Glu Gly Gly Arg Gln Pro Pro Ile Asp Phe Arg Asp
              260   265   270

Val Asp Ile Gly Glu Leu Ser Ser Asp Val Ile Ser Asn Ile Glu Thr
         275   280   285

Phe Asp Val Asn Glu Phe Asp Gln Tyr Leu Pro Pro Asn Gly His Pro
     290   295   300

Gly Val Pro Ala Thr His Gly Gln Val Thr Tyr Thr Gly Ser Tyr Gly
305   310   315   320

Ile Ser Ser Thr Ala Ala Thr Pro Ala Ser Ala Gly His Val Trp Met
                 325   330   335

Ser Lys Gln Gln Ala Pro Pro Pro Pro Gln Gln Pro Pro Gln Ala
              340   345   350

Pro Pro Ala Pro Gln Ala Pro Pro Gln Pro Gln Ala Ala Pro Pro Gln
         355   360   365

Gln Pro Ala Ala Pro Pro Gln Gln Pro Gln Ala His Thr Leu Thr Thr
    370   375   380

Leu Ser Ser Glu Pro Gly Gln Ser Gln Arg Thr His Ile Lys Thr Glu
385   390   395   400

Gln Leu Ser Pro Ser His Tyr Ser Glu Gln Gln Gln His Ser Pro Gln
            405   410   415

Gln Ile Ala Tyr Ser Pro Phe Asn Leu Pro His Tyr Ser Pro Ser Tyr
            420   425   430

Pro Pro Ile Thr Arg Ser Gln Tyr Asp Tyr Thr Asp His Gln Asn Ser
    435   440   445

Ser Ser Tyr Tyr Ser His Ala Ala Gly Gln Gly Thr Gly Leu Tyr Ser
    450   455   460

Thr Phe Thr Tyr Met Asn Pro Ala Gln Arg Pro Met Tyr Thr Pro Ile
465   470   475   480

Ala Asp Thr Ser Gly Val Pro Ser Ile Pro Gln Thr His Ser Pro Gln
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His Trp Glu Gln Pro Val Tyr Thr Gln Leu Thr Arg Pro
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cgcccgggcc cggctcagcc aggccccgcg gtgagcc atg att cgc ctc ggg gct     175
                                        Met Ile Arg Leu Gly Ala
                                         1               5

ccc cag tcg ctg gtg ctg ctg acg ctg ctc gtc gcc gct gtc ctt cgg     223
Pro Gln Ser Leu Val Leu Leu Thr Leu Leu Val Ala Ala Val Leu Arg
             10               15               20

tgt cag ggc cag gat gtc cag gag gct ggc agc tgt gtg cag gat ggg     271
Cys Gln Gly Gln Asp Val Gln Glu Ala Gly Ser Cys Val Gln Asp Gly
         25               30               35

cag agg tat aat gat aag gat gtg tgg aag ccg gag ccc tgc cgg atc     319
Gln Arg Tyr Asn Asp Lys Asp Val Trp Lys Pro Glu Pro Cys Arg Ile
     40               45               50

tgt gtc tgt gac act ggg act gtc ctc tgc gac gac ata atc tgt gaa     367
Cys Val Cys Asp Thr Gly Thr Val Leu Cys Asp Asp Ile Ile Cys Glu
55               60               65               70
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gas gtg aaa gac tgc ctc agc cct gag atc ccc ttc gga gag tgc tgc   415
Asp Val Lys Asp Cys Leu Ser Pro Glu Ile Pro Phe Gly Glu Cys Cys
                 75   80   85

ccc atc tgc cca act gac ctc gca act gcc agt ggg caa cca gga cca   463
Pro Ile Cys Pro Thr Asp Leu Ala Thr Ala Ser Gly Gln Pro Gly Pro
                 90   95   100

aag gga cag aaa gga gaa cct gga gac atc aag gat att gta gga ccc   511
Lys Gly Gln Lys Gly Glu Pro Gly Asp Ile Lys Asp Ile Val Gly Pro
            105   110   115

aaa gga cct cct ggg cct cag gga cct gca ggg gaa caa gga ccc aga   559
Lys Gly Pro Pro Gly Pro Gln Gly Pro Ala Gly Glu Gln Gly Pro Arg
        120   125   130

agg gat cgt ggt gac aaa ggt gaa aaa ggt gcc cct gga cct cgt ggc   607
Gly Asp Arg Gly Asp Lys Gly Glu Lys Gly Ala Pro Gly Pro Arg Gly
    135   140   145   150

aga gat gga gaa cct ggg acc cct gga aat cct ggc ccc cct ggt cct   655
Arg Asp Gly Glu Pro Gly Thr Pro Gly Asn Pro Gly Pro Pro Gly Pro
                155   160   165

ccc ggc ccc cct ggt ccc cct ggt ctt ggt gga aac ttt gct gcc cag   703
Pro Gly Pro Pro Gly Pro Pro Gly Leu Gly Gly Asn Phe Ala Ala Gln
            170   175   180

atg gct gga gga ttt gat gaa aag gct ggt gac gcc cag ttg gga gta   751
Met Ala Gly Gly Phe Asp Glu Lys Ala Gly Asp Ala Gln Leu Gly Val
        185   190   195

atg caa gga cca atg ggc ccc atg gga cct cga ggc cct cca ggc cct   799
Met Gln Gly Pro Met Gly Pro Met Gly Pro Arg Gly Pro Pro Gly Pro
    200   205   210

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gga ggt gct cct ggg cct caa gga ttt caa ggc aat cct ggt gaa cct      847
Ala Gly Ala Pro Gly Pro Gln Gly Phe Gln Gly Asn Pro Gly Glu Pro
215                 220                 225                 230

ggt gaa cct ggt gtc tct ggt ccc atg ggt ccc cgt ggt cct cct ggt      895
Gly Glu Pro Gly Val Ser Gly Pro Met Gly Pro Arg Gly Pro Pro Gly
                235                 240                 245

ccc cct gga aag cct ggt gat gat ggt gaa gct gga aaa cct gga aaa      943
Pro Pro Gly Lys Pro Gly Asp Asp Gly Glu Ala Gly Lys Pro Gly Lys
                250                 255                 260

gct ggt gaa agg ggt ccg cct ggt cct cag ggt gct cgt ggt ttc cca      991
Ala Gly Glu Arg Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Phe Pro
                265                 270                 275

gga acc cca ggc ctt cct ggt gtc aaa ggt cac aga ggt tat cca ggc      1039
Gly Thr Pro Gly Leu Pro Gly Val Lys Gly His Arg Gly Tyr Pro Gly
        280                 285                 290

ctg gac ggt gct aag gga gag gcg ggt gct cct ggt gtg aag ggt gag      1087
Leu Asp Gly Ala Lys Gly Glu Ala Gly Ala Pro Gly Val Lys Gly Glu
295                 300                 305                 310

agt ggt tcc ccg ggt gag aac gga tct ccg ggc cca atg ggt cct cgt      1135
Ser Gly Ser Pro Gly Glu Asn Gly Ser Pro Gly Pro Met Gly Pro Arg
                315                 320                 325

ggc ctg cct ggt gaa aga gga cgg act ggc cct gct ggc gct gcg ggt      1183
Gly Leu Pro Gly Glu Arg Gly Arg Thr Gly Pro Ala Gly Ala Ala Gly
                330                 335                 340

gcc cga ggc aac gat ggt cag cca ggc ccc gca ggt cct ccg ggt cct      1231
Ala Arg Gly Asn Asp Gly Gln Pro Gly Pro Ala Gly Pro Pro Gly Pro
        345                 350                 355
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gtc ggt cct gct ggt ggt cct ggc ttc cct ggt gct cct gga gcc aag      1279
Val Gly Pro Ala Gly Gly Pro Gly Phe Pro Gly Ala Pro Gly Ala Lys
     360                 365                 370

ggt gaa gcc ggc ccc act ggt gcc cgt ggt cct gaa ggt gct caa ggt      1327
Gly Glu Ala Gly Pro Thr Gly Ala Arg Gly Pro Glu Gly Ala Gln Gly
375                 380                 385                 390

cct cgc ggt gaa cct ggt act cct ggg tcc cct ggg cct gct ggt gcc      1375
Pro Arg Gly Glu Pro Gly Thr Pro Gly Ser Pro Gly Pro Ala Gly Ala
                    395                 400                 405

tcc ggt aac cct gga aca gat gga att cct gga gcc aaa gga tct gct      1423
Ser Gly Asn Pro Gly Thr Asp Gly Ile Pro Gly Ala Lys Gly Ser Ala
         410                 415                 420

ggt gct cct ggc att gct ggt gct cct ggc ttc cct ggg cca cgg ggt      1471
Gly Ala Pro Gly Ile Ala Gly Ala Pro Gly Phe Pro Gly Pro Arg Gly
     425                 430                 435

cct cct ggc cct caa ggt gca act ggt cct ctg ggc ccg aaa ggt cag      1519
Pro Pro Gly Pro Gln Gly Ala Thr Gly Pro Leu Gly Pro Lys Gly Gln
     440                 445                 450

acg ggt gaa cct ggt att gct ggc ttc aaa ggt gaa caa ggc ccc aag      1567
Thr Gly Glu Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys
455                 460                 465                 470

gga gaa cct ggc cct gct ggc ccc cag gga gcc cct gga ccc gct ggt      1615
Gly Glu Pro Gly Pro Ala Gly Pro Gln Gly Ala Pro Gly Pro Ala Gly
                    475                 480                 485

gaa gaa ggc aag aga ggt gcc cgt gga gag cct ggt ggc gtt ggg ccc      1663
Glu Glu Gly Lys Arg Gly Ala Arg Gly Glu Pro Gly Gly Val Gly Pro
         490                 495                 500
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atc ggt ccc cct gga gaa aga ggt gct ccc gga aac cga ggt ttc cca    1711
Ile Gly Pro Pro Gly Glu Arg Gly Ala Pro Gly Asn Arg Gly Phe Pro
        505                 510                 515

ggt caa gat ggt ctg gca ggt ccc aag gga gcc cct gga gag cga ggg    1759
Gly Gln Asp Gly Leu Ala Gly Pro Lys Gly Ala Pro Gly Glu Arg Gly
    520                 525                 530

ccc agt ggt ctt gct ggc ccc aag gga gcc aac ggt gac cct ggc cgt    1807
Pro Ser Gly Leu Ala Gly Pro Lys Gly Ala Asn Gly Asp Pro Gly Arg
535                 540                 545                 550

cct gga gaa cct ggc ctt cct gga gcc cgg ggt ctc act ggc cgc cct    1855
Pro Gly Glu Pro Gly Leu Pro Gly Ala Arg Gly Leu Thr Gly Arg Pro
                555                 560                 565

ggt gat gct ggt cct caa ggc aaa gtt ggc cct tct gga gcc cct ggt    1903
Gly Asp Ala Gly Pro Gln Gly Lys Val Gly Pro Ser Gly Ala Pro Gly
                570                 575                 580

gaa gat ggt cgt cct gga cct cca ggt cct cag ggg gct cgt ggg cag    1951
Glu Asp Gly Arg Pro Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Gln
        585                 590                 595

cct ggt gtc atg ggt ttc cct ggc ccc aaa ggt gcc aac ggt gag cct    1999
Pro Gly Val Met Gly Phe Pro Gly Pro Lys Gly Ala Asn Gly Glu Pro
        600                 605                 610

ggc aaa gct ggt gag aag gga ctg cct ggt gct cct ggt ctg agg ggt    2047
Gly Lys Ala Gly Glu Lys Gly Leu Pro Gly Ala Pro Gly Leu Arg Gly
615                 620                 625                 630

ctt cct ggc aaa gat ggt gag aca ggt gct gca gga ccc cct ggc cct    2095
Leu Pro Gly Lys Asp Gly Glu Thr Gly Ala Ala Gly Pro Pro Gly Pro
                635                 640                 645
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gct gga cct gct ggt gaa cga ggc gag cag ggt gct cct ggg cca tct   2143
Ala Gly Pro Ala Gly Glu Arg Gly Glu Gln Gly Ala Pro Gly Pro Ser
            650   655   660

ggg ttc cag gga ctt cct ggc cct cct ggt ccc cca ggt gaa ggt gga   2191
Gly Phe Gln Gly Leu Pro Gly Pro Pro Gly Pro Pro Gly Glu Gly Gly
        665   670   675

aaa cca ggt gac cag ggt gtt ccc ggt gaa gct gga gcc cct ggc ctc   2239
Lys Pro Gly Asp Gln Gly Val Pro Gly Glu Ala Gly Ala Pro Gly Leu
    680   685   690

gtg ggt ccc agg ggt gaa cga ggt ttc cca ggt gaa cgt ggc tct ccc   2287
Val Gly Pro Arg Gly Glu Arg Gly Phe Pro Gly Glu Arg Gly Ser Pro
685   700   705   710

ggt gcc cag ggc ctc cag ggt ccc cgt ggc ctc ccc ggc act cct ggc   2335
Gly Ala Gln Gly Leu Gln Gly Pro Arg Gly Leu Pro Gly Thr Pro Gly
            715   720   725

act gat ggt ccc aaa ggt gca tct ggc cca gca ggc ccc cct ggc gca   2383
Thr Asp Gly Pro Lys Gly Ala Ser Gly Pro Ala Gly Pro Pro Gly Ala
            730   735   740

cag ggc cct cca ggt ctt cag gga atg cct ggc gag agg gga gca gct   2431
Gln Gly Pro Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly Ala Ala
        745   750   755

ggt atc gct ggg ccc aaa ggc gac agg ggt gac gtt ggt gag aaa ggc   2479
Gly Ile Ala Gly Pro Lys Gly Asp Arg Gly Asp Val Gly Glu Lys Gly
    760   765   770

cct gag gga gcc cct gga aag gat ggt gga cga ggc ctg aca ggt ccc   2527
Pro Glu Gly Ala Pro Gly Lys Asp Gly Gly Arg Gly Leu Thr Gly Pro
775   780   785   790

att ggc ccc cct ggc cca gct ggt gct aac ggc gag aag gga gaa gtt 2575
Ile Gly Pro Pro Gly Pro Ala Gly Ala Asn Gly Glu Lys Gly Glu Val
                795   800   805

gga cct cct ggt cct gca gga agt gct ggt gct cgt ggc gct ccg ggt 2623
Gly Pro Pro Gly Pro Ala Gly Ser Ala Gly Ala Arg Gly Ala Pro Gly
             810   815   820

gaa cgt gga gag act ggc ccc ccc gga cca gcg gga ttt gct ggg cct 2671
Glu Arg Gly Glu Thr Gly Pro Pro Gly Pro Ala Gly Phe Ala Gly Pro
          825   830   835

cct ggt gct gat ggc cag cct ggg gcc aag ggt gag caa gga gag gcc 2719
Pro Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly Glu Gln Gly Glu Ala
       840   845   850

ggc cag aaa ggc gat gct ggt gcc cct ggt cct cag ggc ccc tct gga 2767
Gly Gln Lys Gly Asp Ala Gly Ala Pro Gly Pro Gln Gly Pro Ser Gly
855   860   865   870

gca cct ggg cct cag ggt cct act gga gtg act ggt cct aaa gga gcc 2815
Ala Pro Gly Pro Gln Gly Pro Thr Gly Val Thr Gly Pro Lys Gly Ala
             875   880   885

cga ggt gcc caa ggc ccc ccg gga gcc act gga ttc cct gga gct gct 2863
Arg Gly Ala Gln Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly Ala Ala
          890   895   900

ggc cgc gtt gga ccc cca ggc tcc aat ggc aac cct gga ccc cct ggt 2911
Gly Arg Val Gly Pro Pro Gly Ser Asn Gly Asn Pro Gly Pro Pro Gly
       905   910   915

ccc cct ggt cct tct gga aaa gat ggt ccc aaa ggt gct cga gga gac 2959
Pro Pro Gly Pro Ser Gly Lys Asp Gly Pro Lys Gly Ala Arg Gly Asp
       920   925   930

agc ggc ccc cct ggc cgu gct ggt gaa ccc ggc ctc caa ggt cct gct   3007
Ser Gly Pro Pro Gly Arg Ala Gly Glu Pro Gly Leu Gln Gly Pro Ala
935   940   945   950

gga ccc cct ggc gag aag gga gag cct gga gat gac ggt ccc tct ggt   3055
Gly Pro Pro Gly Glu Lys Gly Glu Pro Gly Asp Asp Gly Pro Ser Gly
                    955   960   965

gcc gaa ggt cca cca ggt ccc cag ggt ctg gct ggt cag aga ggc atc   3103
Ala Glu Gly Pro Pro Gly Pro Gln Gly Leu Ala Gly Gln Arg Gly Ile
                    970   975   980

gtc ggt ctg cct ggg caa cgt ggt gag aga gga ttc cct ggc ttg cct   3151
Val Gly Leu Pro Gly Gln Arg Gly Glu Arg Gly Phe Pro Gly Leu Pro
        985   990   995

ggc cca tcg ggt gag ccc ggc aag cag ggt gct cct gga gca tct   3196
Gly Pro Ser Gly Glu Pro Gly Lys Gln Gly Ala Pro Gly Ala Ser
    1000   1005   1010

gga gac aga ggt cct cct ggc ccc gtg ggt gat cct cct ggc ctg acg   3241
Gly Asp Arg Gly Pro Pro Gly Pro Val Gly Asp Pro Pro Gly Leu Thr
    1015   1020   1025

ggt cct gca ggt gaa ccc gga cga gag gga agc ccc ggt gct gat   3286
Gly Pro Ala Gly Glu Pro Gly Arg Glu Gly Ser Pro Gly Ala Asp
    1030   1035   1040

ggg ccc cct ggc aga gat ggc gct gct gga gtc aag ggt gat cgt   3331
Gly Pro Pro Gly Arg Asp Gly Ala Ala Gly Val Lys Gly Asp Arg
    1045   1050   1055

ggt gag act ggt gct gtg gga gct cct gga gcc cct ggg ccc cct   3376
Gly Glu Thr Gly Ala Val Gly Ala Pro Gly Ala Pro Gly Pro Pro
    1060   1065   1070

ggc tcc cct ggc ccc gct ggt cca act ggc aag caa gga gac aga   3421
Gly Ser Pro Gly Pro Ala Gly Pro Thr Gly Lys Gln Gly Asp Arg
　　1075　　　　　　　　　1080　　　　　　　　1085

gga gaa gct ggt gca caa ggc ccc atg gga ccc tca gga cca gct   3466
Gly Glu Ala Gly Ala Gln Gly Pro Met Gly Pro Ser Gly Pro Ala
　　1090　　　　　　　　　1095　　　　　　　　1100

gga gcc cgg gga atc cag ggt cct caa ggc ccc aga ggt gac aaa   3511
Gly Ala Arg Gly Ile Gln Gly Pro Gln Gly Pro Arg Gly Asp Lys
　　1105　　　　　　　　　1110　　　　　　　　1115

gga gag gct gga gag cct ggc gag aga ggc ctg aag gga cac cgt   3556
Gly Glu Ala Gly Glu Pro Gly Glu Arg Gly Leu Lys Gly His Arg
　　1120　　　　　　　　　1125　　　　　　　　1130

ggc ttc act ggt ctg cag ggt ctg ccc ggc cct cct ggt cct tct   3601
Gly Phe Thr Gly Leu Gln Gly Leu Pro Gly Pro Pro Gly Pro Ser
　　1135　　　　　　　　　1140　　　　　　　　1145

gga gac caa ggt gct tct ggt cct gct gct cct tct ggc cct aga   3646
Gly Asp Gln Gly Ala Ser Gly Pro Ala Gly Pro Ser Gly Pro Arg
　　1150　　　　　　　　　1155　　　　　　　　1160

ggt cct cct ggc ccc gtc ggt ccc tct gca aaa gat ggt gct aat   3691
Gly Pro Pro Gly Pro Val Gly Pro Ser Gly Lys Asp Gly Ala Asn
　　1165　　　　　　　　　1170　　　　　　　　1175

gga atc cct ggc ccc att ggg cct cct ggt ccc cgt gga cga tca   3736
Gly Ile Pro Gly Pro Ile Gly Pro Pro Gly Pro Arg Gly Arg Ser
　　1180　　　　　　　　　1185　　　　　　　　1190

ggc gaa acc ggt cct gct ggt cct cct gga aat cct ggg ccc cct   3781
Gly Glu Thr Gly Pro Ala Gly Pro Pro Gly Asn Pro Gly Pro Pro
　　1195　　　　　　　　　1200　　　　　　　　1205

ggt cct cca ggt ccc cct ggc cct ggc atc gac atg tcc gcc ttt   3826
Gly Pro Pro Gly Pro Pro Gly Pro Gly Ile Asp Met Ser Ala Phe
    1210   1215   1220

gct ggc tta ggc ccg aga gag aag ggc ccc gac ccc ctg cag tac   3871
Ala Gly Leu Gly Pro Arg Glu Lys Gly Pro Asp Pro Leu Gln Tyr
    1225   1230   1235

atg cgg gcc gac cag gca gcc ggt ggc ctg aga cag cat gac gcc   3916
Met Arg Ala Asp Gln Ala Ala Gly Gly Leu Arg Gln His Asp Ala
    1240   1245   1250

gag gtg gat gcc aca ctc aag tcc ctc aac aac cag att gag agc   3961
Glu Val Asp Ala Thr Leu Lys Ser Leu Asn Asn Gln Ile Glu Ser
    1255   1260   1265

atc cgc agc ccc gag ggc tcc cgc aag aac cct gct cgc acc tgc   4006
Ile Arg Ser Pro Glu Gly Ser Arg Lys Asn Pro Ala Arg Thr Cys
    1270   1275   1280

aga gac ctg aaa ctc tgc cac cct gag tgg aag agt gga gac tac   4051
Arg Asp Leu Lys Leu Cys His Pro Glu Trp Lys Ser Gly Asp Tyr
    1285   1290   1295

tgg att gac ccc aac caa ggc tgc acc ttg gac gcc atg aag gtt   4096
Trp Ile Asp Pro Asn Gln Gly Cys Thr Leu Asp Ala Met Lys Val
    1300   1305   1310

ttc tgc aac atg gag act ggc gag act tgc gtc tac ccc aat cca   4141
Phe Cys Asn Met Glu Thr Gly Glu Thr Cys Val Tyr Pro Asn Pro
    1315   1320   1325

gca aac gtt ccc aag aag aac tgg tgg agc agc aag agc aag gag   4186
Ala Asn Val Pro Lys Lys Asn Trp Trp Ser Ser Lys Ser Lys Glu
    1330   1335   1340

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aag aaa  cac atc tgg ttt gga  gaa acc atc aat ggt  ggc ttc cat   4231
Lys Lys  His Ile Trp Phe Gly  Glu Thr Ile Asn Gly  Gly Phe His
     1345                1350                1355

ttc agc  tat gga gat gac aat  ctg gct ccc aac act  gcc aac gtc   4276
Phe Ser  Tyr Gly Asp Asp Asn  Leu Ala Pro Asn Thr  Ala Asn Val
     1360                1365                1370

cag atg  acc ttc cta cgc ctg  ctg tcc acg gaa ggc  tcc cag aac   4321
Gln Met  Thr Phe Leu Arg Leu  Leu Ser Thr Glu Gly  Ser Gln Asn
     1375                1380                1385

atc acc  tac cac tgc aag aac  agc att gcc tat ctg  gac gaa gca   4366
Ile Thr  Tyr His Cys Lys Asn  Ser Ile Ala Tyr Leu  Asp Glu Ala
     1390                1395                1400

gct ggc  aac ctc aag aag gcc  ctg ctc atc cag ggc  tcc aat gac   4411
Ala Gly  Asn Leu Lys Lys Ala  Leu Leu Ile Gln Gly  Ser Asn Asp
     1405                1410                1415

gtg gag  atc cgg gca gag ggc  aat agc agg ttc acg  tac act gcc   4456
Val Glu  Ile Arg Ala Glu Gly  Asn Ser Arg Phe Thr  Tyr Thr Ala
     1420                1425                1430

ctg aag  gat ggc tgc acg aaa  cat acc ggt aag tgg  ggc aag act   4501
Leu Lys  Asp Gly Cys Thr Lys  His Thr Gly Lys Trp  Gly Lys Thr
     1435                1440                1445

gtt atc  gag tac cgg tca cag  aag acc tca cgc ctc  ccc atc att   4546
Val Ile  Glu Tyr Arg Ser Gln  Lys Thr Ser Arg Leu  Pro Ile Ile
     1450                1455                1460

gac att  gca ccc atg gac ata  gga ggc ccc gag cag  gaa ttc ggt   4591
Asp Ile  Ala Pro Met Asp Ile  Gly Gly Pro Glu Gln  Glu Phe Gly
     1465                1470                1475
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gtg gac ata gga ccg gtc tgc ttc ttg taa aaacctgaac ccagaaacaa   4641
Val Asp Ile Gly Pro Val Cys Phe Leu
   1480   1485

cacaatccgt tgcaaaccca aaggacccaa gtactttcca atctcagtca ctctaggact   4701

ctgcactgaa tggctgacct gacctgatgt ccattcatcc caccctctca cagttcggac   4761

ttttctcccc tctctttcta agagacctga actgggcaga ctgcaaaata aaatctcggt   4821

gttctatttta tttattgtct tcctgtaaga cctcgggtc aaggcagagg caggaaacta   4881

actggtgtga gtcaaatgcc ccctgagtga ctgccccaag cccaggccag aagacctccc   4941

ttcaggtgcc gggcgcagga actgtgtgtg tcctacacaa tggtgctatt ctgtgtcaaa   5001

cacctctgta tttttttaaaa catcaattga tattaaaaat gaaaagatta ttggaaagt   5060

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<212> PRT
<213> Homo sapiens

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Met Ile Arg Leu Gly Ala Pro Gln Ser Leu Val Leu Leu Thr Leu Leu
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Val Ala Ala Val Leu Arg Cys Gln Gly Gln Asp Val Gln Glu Ala Gly
     20   25   30

Ser Cys Val Gln Asp Gly Gln Arg Tyr Asn Asp Lys Asp Val Trp Lys
    35   40   45

Pro Glu Pro Cys Arg Ile Cys Val Cys Asp Thr Gly Thr Val Leu Cys
    50   55   60

Asp Asp Ile Ile Cys Glu Asp Val Lys Asp Cys Leu Ser Pro Glu Ile
65   70   75   80

Pro Phe Gly Glu Cys Cys Pro Ile Cys Pro Thr Asp Leu Ala Thr Ala
                85   90   95

Ser Gly Gln Pro Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly Asp Ile
            100   105   110

Lys Asp Ile Val Gly Pro Lys Gly Pro Pro Gly Pro Gln Gly Pro Ala
        115   120   125

Gly Glu Gln Gly Pro Arg Gly Asp Arg Gly Asp Lys Gly Glu Lys Gly
    130   135   140

Ala Pro Gly Pro Arg Gly Arg Asp Gly Glu Pro Gly Thr Pro Gly Asn
145   150   155   160

Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Leu Gly
            165   170   175

Gly Asn Phe Ala Ala Gln Met Ala Gly Gly Phe Asp Glu Lys Ala Gly
            180   185   190

Gly Ala Gln Leu Gly Val Met Gln Gly Pro Met Gly Pro Met Gly Pro
        195   200   205

Arg Gly Pro Pro Gly Pro Ala Gly Ala Pro Gly Pro Gln Gly Phe Gln
        210   215   220

Gly Asn Pro Gly Glu Pro Gly Glu Pro Gly Val Ser Gly Pro Met Gly
225   230   235   240

Pro Arg Gly Pro Pro Gly Pro Pro Gly Lys Pro Gly Asp Asp Gly Glu
                245   250   255

Ala Gly Lys Pro Gly Lys Ala Gly Glu Arg Gly Pro Pro Gly Pro Gln
                260   265   270

Gly Ala Arg Gly Phe Pro Gly Thr Pro Gly Leu Pro Gly Val Lys Gly
        275   280   285

His Arg Gly Tyr Pro Gly Leu Asp Gly Ala Lys Gly Glu Ala Gly Ala
        290   295   300

Pro Gly Val Lys Gly Glu Ser Gly Ser Pro Gly Glu Asn Gly Ser Pro
305   310   315   320

Gly Pro Met Gly Pro Arg Gly Leu Pro Gly Glu Arg Gly Arg Thr Gly
                325   330   335

Pro Ala Gly Ala Ala Gly Ala Arg Gly Asn Asp Gly Gln Pro Gly Pro
          340   345   350

Ala Gly Pro Pro Gly Pro Val Gly Pro Ala Gly Gly Pro Gly Phe Pro
          355   360   365

Gly Ala Pro Gly Ala Lys Gly Glu Ala Gly Pro Thr Gly Ala Arg Gly
     370   375   380

Pro Glu Gly Ala Gln Gly Pro Arg Gly Glu Pro Gly Thr Pro Gly Ser
385   390   395   400

Pro Gly Pro Ala Gly Ala Ser Gly Asn Pro Gly Thr Asp Gly Ile Pro
          405   410   415

Gly Ala Lys Gly Ser Ala Gly Ala Pro Gly Ile Ala Gly Ala Pro Gly
          420   425   430

Phe Pro Gly Pro Arg Gly Pro Pro Gly Pro Gln Gly Ala Thr Gly Pro
          435   440   445

Leu Gly Pro Lys Gly Gln Thr Gly Glu Pro Gly Ile Ala Gly Phe Lys
     450   455   460

Gly Glu Gln Gly Pro Lys Gly Glu Pro Gly Pro Ala Gly Pro Gln Gly
465   470   475   480

Ala Pro Gly Pro Ala Gly Glu Glu Gly Lys Arg Gly Ala Arg Gly Glu
                485   490   495

Pro Gly Gly Val Gly Pro Ile Gly Pro Pro Gly Glu Arg Gly Ala Pro
            500   505   510

Gly Asn Arg Gly Phe Pro Gly Gln Asp Gly Leu Ala Gly Pro Lys Gly
        515   520   525

Ala Pro Gly Glu Arg Gly Pro Ser Gly Leu Ala Gly Pro Lys Gly Ala
    530   535   540

Asn Gly Asp Pro Gly Arg Pro Gly Glu Pro Gly Leu Pro Gly Ala Arg
545   550   555   560

Gly Leu Thr Gly Arg Pro Gly Asp Ala Gly Pro Gln Gly Lys Val Gly
                565   570   575

Pro Ser Gly Ala Pro Gly Glu Asp Gly Arg Pro Gly Pro Pro Gly Pro
            580   585   590

Gln Gly Ala Arg Gly Gln Pro Gly Val Met Gly Phe Pro Gly Pro Lys
        595   600   605

Gly Ala Asn Gly Glu Pro Gly Lys Ala Gly Glu Lys Gly Leu Pro Gly
    610   615   620

Ala Pro Gly Leu Arg Gly Leu Pro Gly Lys Asp Gly Glu Thr Gly Ala
625   630   635   640

Ala Gly Pro Pro Gly Pro Ala Gly Pro Ala Gly Glu Arg Gly Glu Gln
                    645   650   655

Gly Ala Pro Gly Pro Ser Gly Phe Gln Gly Leu Pro Gly Pro Pro Gly
            660   665   670

Pro Pro Gly Glu Gly Gly Lys Pro Gly Asp Gln Gly Val Pro Gly Glu
        675   680   685

Ala Gly Ala Pro Gly Leu Val Gly Pro Arg Gly Glu Arg Gly Phe Pro
    690   695   700

Gly Glu Arg Gly Ser Pro Gly Ala Gln Gly Leu Gln Gly Pro Arg Gly
705   710   715   720

Leu Pro Gly Thr Pro Gly Thr Asp Gly Pro Lys Gly Ala Ser Gly Pro
                725   730   735

Ala Gly Pro Pro Gly Ala Gln Gly Pro Pro Gly Leu Gln Gly Met Pro
            740   745   750

Gly Glu Arg Gly Ala Ala Gly Ile Ala Gly Pro Lys Gly Asp Arg Gly
    755   760   765

Asp Val Gly Glu Lys Gly Pro Glu Gly Ala Pro Gly Lys Asp Gly Gly
   770   775   780

Arg Gly Leu Thr Gly Pro Ile Gly Pro Pro Gly Pro Ala Gly Ala Asn
785   790   795   800

Gly Glu Lys Gly Glu Val Gly Pro Pro Gly Pro Ala Gly Ser Ala Gly
         805   810   815

Ala Arg Gly Ala Pro Gly Glu Arg Gly Glu Thr Gly Pro Pro Gly Pro
        820   825   830

Ala Gly Phe Ala Gly Pro Pro Gly Ala Asp Gly Gln Pro Gly Ala Lys
     835   840   845

Gly Glu Gln Gly Glu Ala Gly Gln Lys Gly Asp Ala Gly Ala Pro Gly
  850   855   860

Pro Gln Gly Pro Ser Gly Ala Pro Gly Pro Gln Gly Pro Thr Gly Val
865   870   875   880

Thr Gly Pro Lys Gly Ala Arg Gly Ala Gln Gly Pro Pro Gly Ala Thr
        885   890   895

Gly Phe Pro Gly Ala Ala Gly Arg Val Gly Pro Pro Gly Ser Asn Gly
     900   905   910

Asn Pro Gly Pro Pro Gly Pro Pro Gly Pro Ser Gly Lys Asp Gly Pro
          915   920   925

Lys Gly Ala Arg Gly Asp Ser Gly Pro Pro Gly Arg Ala Gly Glu Pro
     930   935   940

Gly Leu Gln Gly Pro Ala Gly Pro Pro Gly Glu Lys Gly Glu Pro Gly
945   950   955   960

Asp Asp Gly Pro Ser Gly Ala Glu Gly Pro Pro Gly Pro Gln Gly Leu
               965   970   975

Ala Gly Gln Arg Gly Ile Val Gly Leu Pro Gly Gln Arg Gly Glu Arg
               980   985   990

Gly Phe Pro Gly Leu Pro Gly Pro Ser Gly Glu Pro Gly Lys Gln Gly
          995   1000   1005

Ala Pro Gly Ala Ser Gly Asp Arg Gly Pro Pro Gly Pro Val Gly
     1010   1015   1020

Pro Pro Gly Leu Thr Gly Pro Ala Gly Glu Pro Gly Arg Glu Gly
     1025   1030   1035

Ser Pro Gly Ala Asp Gly Pro Pro Gly Arg Asp Gly Ala Ala Gly
     1040   1045   1050

Val Lys Gly Asp Arg Gly Glu Thr Gly Ala Val Gly Ala Pro Gly
    1055   1060   1065

Ala Pro Gly Pro Pro Gly Ser Pro Gly Pro Ala Gly Pro Thr Gly
    1070   1075   1080

Lys Gln Gly Asp Arg Gly Glu Ala Gly Ala Gln Gly Pro Met Gly
    1085   1090   1095

Pro Ser Gly Pro Ala Gly Ala Arg Gly Ile Gln Gly Pro Gln Gly
    1100   1105   1110

Pro Arg Gly Asp Lys Gly Glu Ala Gly Glu Pro Gly Glu Arg Gly
    1115   1120   1125

Leu Lys Gly His Arg Gly Phe Thr Gly Leu Gln Gly Leu Pro Gly
    1130   1135   1140

Pro Pro Gly Pro Ser Gly Asp Gln Gly Ala Ser Gly Pro Ala Gly
    1145   1150   1155

Pro Ser Gly Pro Arg Gly Pro Pro Gly Pro Val Gly Pro Ser Gly
    1160   1165   1170

Lys Asp Gly Ala Asn Gly Ile Pro Gly Pro Ile Gly Pro Pro Gly
    1175   1180   1185

Pro Arg Gly Arg Ser Gly Glu Thr Gly Pro Ala Gly Pro Pro Gly
    1190   1195   1200

Asn Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Gly Ile
    1205   1210   1215

Asp Met Ser Ala Phe Ala Gly Leu Gly Pro Arg Glu Lys Gly Pro
    1220   1225   1230

Asp Pro Leu Gln Tyr Met Arg Ala Asp Gln Ala Ala Gly Gly Leu
    1235   1240   1245

Arg Gln His Asp Ala Glu Val Asp Ala Thr Leu Lys Ser Leu Asn
    1250   1255   1260

Asn Gln Ile Glu Ser Ile Arg Ser Pro Glu Gly Ser Arg Lys Asn
    1265   1270   1275

Pro Ala Arg Thr Cys Arg Asp Leu Lys Leu Cys His Pro Glu Trp
    1280   1285   1290

Lys Ser Gly Asp Tyr Trp Ile Asp Pro Asn Gln Gly Cys Thr Leu
    1295   1300   1305

Asp Ala Met Lys Val Phe Cys Asn Met Glu Thr Gly Glu Thr Cys
    1310   1315   1320

Val Tyr Pro Asn Pro Ala Asn Val Pro Lys Lys Asn Trp Trp Ser
    1325   1330   1335

Ser Lys Ser Lys Glu Lys Lys His Ile Trp Phe Gly Glu Thr Ile
    1340   1345   1350

Asn Gly Gly Phe His Phe Ser Tyr Gly Asp Asp Asn Leu Ala Pro
    1355   1360   1365

Asn Thr Ala Asn Val Gln Met Thr Phe Leu Arg Leu Leu Ser Thr
    1370   1375   1380

Glu Gly Ser Gln Asn Ile Thr Tyr His Cys Lys Asn Ser Ile Ala
    1385   1390   1395

Tyr Leu Asp Glu Ala Ala Gly Asn Leu Lys Lys Ala Leu Leu Ile
    1400   1406   1410

Gln Gly Ser Asn Asp Val Glu Ile Arg Ala Glu Gly Asn Ser Arg
    1415   1420   1425

Phe Thr Tyr Thr Ala Leu Lys Asp Gly Cys Thr Lys His Thr Gly
    1430   1435   1440

Lys Trp Gly Lys Thr Val Ile Glu Tyr Arg Ser Gln Lys Thr Ser
    1445   1450   1456

Arg Leu Pro Ile Ile Asp Ile Ala Pro Met Asp Ile Gly Gly Pro
     1460   1465   1470

Glu Gln Glu Phe Gly Val Asp Ile Gly Pro Val Cys Phe Leu
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atg acc act tta ctc tgg gtt ttc gtg act ctg agg gtc ata act gca   108
Met Thr Thr Leu Leu Trp Val Phe Val Thr Leu Arg Val Ile Thr Ala
1   5   10   15

gct gtc act gta gaa act tca gac cat gac aac tcg ctg agt gtc agc   156
Ala Val Thr Val Glu Thr Ser Asp His Asp Asn Ser Leu Ser Val Ser
             20   25   30

atc cca gaa ccg tcc ccg ctg agg gtc ctc ctg gag acc tcc ctc acc   204
Ile Pro Gln Pro Ser Pro Leu Arg Val Leu Leu Gly Thr Ser Leu Thr
         35   40   45

atc ccc tgc tat ttc atc gac ccc atg gac cct gtg acc acc gcc cct   252

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Ile Pro Cys Tyr Phe Ile Asp Pro Met His Pro Val Thr Thr Ala Pro
     50                  55                  60

tct acc gcc cca ctg gcc cca aga atc aag tgg agc cgt gtg tcc aag      300
Ser Thr Ala Pro Leu Ala Pro Arg Ile Lys Trp Ser Arg Val Ser Lys
 65                  70                  75                  80

gag aag gag gta gtg ctg ctg gtg gcc act gaa ggg cgc gtg cgg gtc      348
Glu Lys Glu Val Val Leu Leu Val Ala Thr Glu Gly Arg Val Arg Val
                 85                  90                  95

aac agt gcc tat cag gac aag gtc tca ctg ccc aac tac ccg gcc atc      396
Asn Ser Ala Tyr Gln Asp Lys Val Ser Leu Pro Asn Tyr Pro Ala Ile
                100                 105                 110

ccc agt gac gcc acc ttg gaa gtc cag agc ctg cgc tcc aat gac tct      444
Pro Ser Asp Ala Thr Leu Glu Val Gln Ser Leu Arg Ser Asn Asp Ser
            115                 120                 125

ggg gtc tac cgc tgc gag gtg atg cat ggc atc gag gac agc gag gcc      492
Gly Val Tyr Arg Cys Glu Val Met His Gly Ile Glu Asp Ser Glu Ala
            130                 135                 140

acc ctg gaa gtc gtg gtg aaa ggc atc gtg ttc cat tac aga gcc atc      540
Thr Leu Glu Val Val Val Lys Gly Ile Val Phe His Tyr Arg Ala Ile
145                 150                 155                 160

tct aca cgc tac acc ctc gac ttt gac agg gcg cag cgg gcc tgc ctg      588
Ser Thr Arg Tyr Thr Leu Asp Phe Asp Arg Ala Gln Arg Ala Cys Leu
                165                 170                 175

cag aac agt gcc atc att gcc acg cct gag cag ctg cag gcc gcc tac      636
Gln Asn Ser Ala Ile Ile Ala Thr Pro Glu Gln Leu Gln Ala Ala Tyr
            180                 185                 190

gaa gac ggc ttc cac cag tgt gac gcc ggc tgg ctg gct gac cag act      684
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Glu Asp Gly Phe His Gln Cys Asp Ala Gly Trp Leu Ala Asp Gln Thr
        195   200   205

gtc aga tac ccc atc cac act ccc cgg gaa ggc tgc tat gga gac aag   732
Val Arg Tyr Pro Ile His Thr Pro Arg Glu Gly Cys Tyr Gly Asp Lys
   210   215   220

gat gag ttt cct ggt gtg agg acg tat ggc atc cga gac acc aac gag   780
Asp Glu Phe Pro Gly Val Arg Thr Tyr Gly Ile Arg Asp Thr Asn Glu
225   230   235   240

acc tat gat gtg tac tgc ttc gcc gag gag atg gag ggt gag gtc ttt   828
Thr Tyr Asp Val Tyr Cys Phe Ala Glu Glu Met Glu Gly Glu Val Phe
                245   250   255

tat gca aca tct cca gag aag ttc acc ttc cag gaa gca gcc aat gag   876
Tyr Ala Thr Ser Pro Glu Lys Phe Thr Phe Gln Glu Ala Ala Asn Glu
                260   265   270

tgc cgg cgg ctg ggt gcc cgg ctg gcc acc acg ggc cac gtc tac ctg   924
Cys Arg Arg Leu Gly Ala Arg Leu Ala Thr Thr Gly His Val Tyr Leu
        275   280   285

gcc tgg cag gct ggc atg gac atg tgc agc gcc ggc tgg ctg gcc gac   972
Ala Trp Gln Ala Gly Met Asp Met Cys Ser Ala Gly Trp Leu Ala Asp
   290   295   300

cgc agc gtg cgc tac ccc atc tcc aag gcc cgg ccc aac tgc ggt ggc   1020
Arg Ser Val Arg Tyr Pro Ile Ser Lys Ala Arg Pro Asn Cys Gly Gly
305   310   315   320

aac ctc ctg ggc gtg agg acc gtc tac gtg cat gcc aac cag acg gga   1068
Asn Leu Leu Gly Val Arg Thr Val Tyr Val His Ala Asn Gln Thr Gly
                325   330   335

tac ccc gac ccc tca tcc cgc tac gac gcc atc tgc tac aca ggt gaa   1116

Tyr Pro Asp Pro Ser Ser Arg Tyr Asp Ala Ile Cys Tyr Thr Gly Glu
            340   345   350

gac ttt gtg gac atc cca gaa aac ttc ttt gga gtg ggg ggt gag gag   1164
Asp Phe Val Asp Ile Pro Glu Asn Phe Phe Gly Val Gly Gly Glu Glu
        355   360   365

gac atc acc gtc cag aca gtg acc tgg cct gac atg gag ctg cca ctg   1212
Asp Ile Thr Val Gln Thr Val Thr Trp Pro Asp Met Glu Leu Pro Leu
    370   375   380

cct cga aac atc act gag ggt gaa gcc cga ggc agc gtg atc ctt acc   1260
Pro Arg Asn Ile Thr Glu Gly Glu Ala Arg Gly Ser Val Ile Leu Thr
385   390   395   400

gta aag ccc atc ttc gag gtc tcc ccc agt ccc ctg gaa ccc gag gag   1308
Val Lys Pro Ile Phe Glu Val Ser Pro Ser Pro Leu Glu Pro Glu Glu
                405   410   415

ccc ttc acg ttt gcc cct gaa ata ggg gcc act gcc ttc gct gag gtt   1356
Pro Phe Thr Phe Ala Pro Glu Ile Gly Ala Thr Ala Phe Ala Glu Val
                420   425   430

gag aat gag act gga gag gcc acc agg ccc tgg ggc ttt ccc aca cct   1404
Glu Asn Glu Thr Gly Glu Ala Thr Arg Pro Trp Gly Phe Pro Thr Pro
            435   440   445

ggc ctg ggc cct gcc acg gca ttc acc agt gag gac ctc gtc gtg cag   1452
Gly Leu Gly Pro Ala Thr Ala Phe Thr Ser Glu Asp Leu Val Val Gln
    460   455   460

gtg acc gct gtc cct ggg cag ccg cat ttg cca ggg ggt gtc gtc ttc   1500
Val Thr Ala Val Pro Gly Gln Pro His Leu Pro Gly Gly Val Val Phe
465   470   475   480

cac tac agc ccg gga ccc acc cgc tac tgg ctg acc ttt gag gag gca   1548

His Tyr Arg Pro Gly Pro Thr Arg Tyr Ser Leu Thr Phe Glu Glu Ala
            485   490   495

cag cag gcc tgc cct agc acg ggg gca gtc att gcc tcg cag gag cag   1596
Gln Gln Ala Cys Pro Gly Thr Gly Ala Val Ile Ala Ser Pro Glu Gln
            500   505   510

ctc cag gcc gcc tac gaa gca ggc tat gag cag tgt gac gcc ggc tgg   1644
Leu Gln Ala Ala Tyr Glu Ala Gly Tyr Glu Gln Cys Asp Ala Gly Trp
            515   520   525

ctg cgg gac cag acc gtc aga tac ccc att gtg agc cca cgg acc cca   1692
Leu Arg Asp Gln Thr Val Arg Tyr Pro Ile Val Ser Pro Arg Thr Pro
       530   535   540

tgc gtg ggt gac aag gac agc agc cca ggg gtc agg acc tat ggc gtg , , 1740
Cys Val Gly Asp Lys Asp Ser Ser Pro Gly Val Arg Thr Tyr Gly Val
545   550   555   560

cgc cca tca aca gag acc tac gat gtc tac tgc ttt gta gac aga ctt   1788
Arg Pro Ser Thr Glu Thr Tyr Asp Val Tyr Cys Phe Val Asp Arg Leu
            565   570   575

gag ggg gag gtg ttc ttc gcc aca cgc ctt gag cag ttc acc ttc cag   1836
Glu Gly Glu Val Phe Phe Ala Thr Arg Leu Glu Gln Phe Thr Phe Gln
            580   585   590

gaa gca ctg gag ttc tgt gaa tct cac aat gcc act gcc acc acg ggc   1884
Glu Ala Leu Glu Phe Cys Glu Ser His Asn Ala Thr Ala Thr Thr Gly
       595   600   605

cag ctc tac gcc gcc tgg agc cgc ggc ctg gac aag tgc tat gcc ggc   1932
Gln Leu Tyr Ala Ala Trp Ser Arg Gly Leu Asp Lys Cys Tyr Ala Gly
       610   615   620

tgg ctg gcc gac ggc agc ctc cgc tac ccc atc gtc acc cca agg cct   1980

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Trp Leu Ala Asp Gly Ser Leu Arg Tyr Pro Ile Val Thr Pro Arg Pro
625                 630             635             640

gcc tgc ggt ggg gac aag cca ggc gtg aga acg gtc tac ctc tac cct    2028
Ala Cys Gly Gly Asp Lys Pro Gly Val Arg Thr Val Tyr Leu Tyr Pro
                645             650             655

aac cag acg ggc ctc cca gac cca ctg tcc agg cac aat gcc ttc tgc    2076
Asn Gln Thr Gly Leu Pro Asp Pro Leu Ser Arg His His Ala Phe Cys
            660             665             670

ttc cga ggc att tca gcg gtt cct tct cca gga gaa gaa gag ggt ggc    2124
Phe Arg Gly Ile Ser Ala Val Pro Ser Pro Gly Glu Glu Glu Gly Gly
        675             680             685

aca ccc aca tca ccc tct ggt gtg gag gag tgg atc gtg acc caa gtg    2172
Thr Pro Thr Ser Pro Ser Gly Val Glu Glu Trp Ile Val Thr Gln Val
    690             695             700

gtt cct ggt gtg gct gct gtc ccc gta gaa gag gag aca act gct gta    2220
Val Pro Gly Val Ala Ala Val Pro Val Glu Glu Glu Thr Thr Ala Val
705             710             715             720

ccc tca ggg gag act act gcc atc cta gag ttc acc acc gag cca gaa    2268
Pro Ser Gly Glu Thr Thr Ala Ile Leu Glu Phe Thr Thr Glu Pro Glu
                725             730             735

aac cag aca gaa tgg gaa cca gcc tat acc cca gtg ggc aca tcc ccg    2316
Asn Gln Thr Glu Trp Glu Pro Ala Tyr Thr Pro Val Gly Thr Ser Pro
            740             745             750

ctg cca ggg atc ctt cct act tgg cct cct act ggc gcc gaa aca gag    2364
Leu Pro Gly Ile Leu Pro Thr Trp Pro Pro Thr Gly Ala Glu Thr Glu
        755             760             765

gaa agt aca gaa ggc cct tct gca act gaa gtg ccc tct gcc tca gag    2412
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Glu Ser Thr Glu Gly Pro Ser Ala Thr Glu Val Pro Ser Ala Ser Glu
       770   775   780

gaa cca tcc ccc tca gag gtg cca ttc ccc tca gag gag cca tcc cac   2460
Glu Pro Ser Pro Ser Glu Val Pro Phe Pro Ser Glu Glu Pro Ser Pro
785   790   795   800

tca gag gaa cca ttc ccc tca gtg agg cca ttc ccc tca gtg gag ctg   2508
Ser Glu Glu Pro Phe Pro Ser Val Arg Pro Phe Pro Ser Val Glu Leu
                805   810   815

ttc ccc tca gag gag cca ttc ccc tcc aag gag cca tcc ccc tca gag   2556
Phe Pro Ser Glu Glu Pro Phe Pro Ser Lys Glu Pro Ser Pro Ser Glu
                820   825   830

gaa cca tca gcc tca gaa gag ccg tat acc cct tca ccc ccc gag ccc   ·2604
Glu Pro Ser Ala Ser Glu Glu Pro Tyr Thr Pro Ser Pro Pro Glu Pro
                835   840   845

agc tgg act gag ctg ccc agc tct ggg gag gaa tct ggg gcc cct gat   2652
Ser Trp Thr Glu Leu Pro Ser Ser Gly Glu Glu Ser Gly Ala Pro Asp
    850   855   860

gtc agt ggt gac ttc aca ggc agt gga gat gtt tca gga cac ctt gac   2700
Val Ser Gly Asp Phe Thr Gly Ser Gly Asp Val Ser Gly His Leu Asp
865   870   875   880

tto agt ggg cag ctg tca ggg gac agg gca agt gga ctg ccc tct gga   2748
Phe Ser Gly Gln Leu Ser Gly Asp Arg Ala Ser Gly Leu Pro Ser Gly
                885   890   895

gac ctg gac tcc agt ggt ctt act tcc aca gtg ggc tca ggc ctg act   2796
Asp Leu Asp Ser Ser Gly Leu Thr Ser Thr Val Gly Ser Gly Leu Thr
                900   905   910

gtg gaa agt gga cta ccc tca ggg gat gaa gag aga att gag tgg ccc   2844

Val Glu Ser Gly Leu Pro Ser Gly Asp Glu Glu Arg Ile Glu Trp Pro
          915   920   925

agc act cct acg gtt ggt gaa ctg ccc tct gga gct gag atc cta gag   2892
Ser Thr Pro Thr Val Gly Glu Leu Pro Ser Gly Ala Glu Ile Leu Glu
     930   935   940

ggc tct gcc tct gga gtt ggg gat ctc agt gga ctt cct tct gga gaa   2940
Gly Ser Ala Ser Gly Val Gly Asp Leu Ser Gly Leu Pro Ser Gly Glu
945   950   955   960

gtt cta gag acc tct gcc tct gga gta gga gac ctc agt ggg ctt cct   2988
Val Leu Glu Thr Ser Ala Ser Gly Val Gly Asp Leu Ser Gly Leu Pro
                    965   970   975

tct gga gaa gtt cta gag acc act gcc cct gga gta gag gac atc agc   3036
Ser Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu Asp Ile Ser
               980   985   990

ggg ctt cct tct gga gaa gtt cta gag acc act gcc cct gga gta gag   3084
Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu
          995   1000   1005

gac atc agc ggg ctt cct tct gga gaa gtt cta gag acc act gcc   3129
Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala
     1010   1015   1020

cct gga gta gag gac atc agc ggg ctt cct tct gga gaa gtt cta   3174
Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu
     1025   1030   1035

gag acc act gcc cct gga gta gag gac atc agc ggg ctt cct tct   3219
Glu Thr Thr Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser
     1040   1045   1050

gga gaa gtt cta gag acc act gcc cct gga gta gag gac atc agc   3264

Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu Asp Ile Ser
     1055   1060   1065

gga ctt cct tct gga gaa gtt cta gag acc gct gcc cct gga gta   3309
Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly Val
     1070   1075   1080

gag gac atc agc ggg ctt cct tct gga gaa gtt cta gag acc gct   3354
Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala
     1085   1090   1095

gcc cct gga gta gag gac atc agc ggg ctt cct tct gga gaa gtt   3399
Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val
     1100   1105   1110

cta gag acc gct gcc cct gga gta gag gac atc agc ggg ctt cct   3444
Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro
     1115   1120   1125

tct gga gaa gtt cta gag acc gct gcc cct gga gta gag gac atc   3489
Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile
     1130   1135   1140

agc ggg ctt cct tct gga gaa gtt cta gag acc gct gcc cct gga   3534
Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly
     1145   1150   1155

gta gag gac atc agc ggg ctt cct tct gga gaa gtt cta gag acc   3579
Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr
     1160   1165   1170

gct gcc cct gga gta gag gac atc agc ggg ctt cct tct gga gaa   3624
Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu
     1175   1180   1185

gtt cta gag acc gct gcc cct gga gta gag gac atc agc ggg ctt   3669

Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu
   1190   1195   1200

cct tct gga gaa gtt cta gag act gct gcc cct gga gta gag gac   3714
Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp
   1205   1210   1215

atc agc ggg ctt cct tct gga gaa gtt cta gag act gct gcc cct   3759
Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro
   1220   1225   1230

gga gta gag gac atc agc ggg ctt cct tct gga gaa gtt cta gag   3804
Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu
   1235   1240   1245

act gct gcc cct gga gta gag gac atc agc ggg ctt cct tct gga   3849
Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly
   1250   1255   1260

gaa gtt cta gag act gct gcc cct gga gta gag gac atc agc ggg   3894
Glu Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly
   1265   1270   1275

ctt cct tct gga gaa gtt cta gag act act gcc cct gga gta gag   3939
Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu
   1280   1285   1290

gag atc agc ggg ctt cct tct gga gaa gtt cta gag act act gcc   3984
Glu Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala
   1295   1300   1305

cct gga gta gat gag atc agt ggg ctt cct tct gga gaa gtt cta   4029
Pro Gly Val Asp Glu Ile Ser Gly Leu Pro Ser Gly Glu Val Leu
   1310   1315   1320

gag act act gcc cct gga gta gag gag atc agc ggg ctt cct tct   4074

```
Glu Thr  Thr Ala Pro Gly Val  Glu Glu Ile Ser Gly  Leu Pro Ser
     1325                 1330                 1335

gga gaa  gtt cta gag act tct  acc tct gcg gta ggg  gac ctc agt          4119
Gly Glu  Val Leu Glu Thr Ser  Thr Ser Ala Val Gly  Asp Leu Ser
     1340                 1345                 1350

gga ctt  cct tct gga gga gaa  gtt cta gag att tct  gtc tct gga          4164
Gly Leu  Pro Ser Gly Gly Glu  Val Leu Glu Ile Ser  Val Ser Gly
     1355                 1360                 1365

gta gag  gac atc agt ggg ctt  cct tct gga gag gtt  gta gag act          4209
Val Glu  Asp Ile Ser Gly Leu  Pro Ser Gly Glu Val  Val Glu Thr
     1370                 1375                 1380

tct gcc  tct gga ata gag gat  gtc agt gaa ctt cct  tca gga gaa          4254
Ser Ala  Ser Gly Ile Glu Asp  Val Ser Glu Leu Pro  Ser Gly Glu
     1385                 1390                 1395

ggt cta  gag acc tct gct tct  gga gta gag gac ctc  agc agg ctc          4299
Gly Leu  Glu Thr Ser Ala Ser  Gly Val Glu Asp Leu  Ser Arg Leu
     1400                 1405                 1410

cct tct  gga gaa gaa gtt cta  gag att tct gcc tct  gga ttt ggg          4344
Pro Ser  Gly Glu Glu Val Leu  Glu Ile Ser Ala Ser  Gly Phe Gly
     1415                 1420                 1425

gac ctc  agt gga gtt cct tct  gga gga gaa ggt cta  gag acc tct          4389
Asp Leu  Ser Gly Val Pro Ser  Gly Gly Glu Gly Leu  Glu Thr Ser
     1430                 1435                 1440

gct tct  gaa gta ggg act gac  ctc agt ggg ctt cct  tct gga agg          4434
Ala Ser  Glu Val Gly Thr Asp  Leu Ser Gly Leu Pro  Ser Gly Arg
     1445                 1450                 1455

gag ggt  cta gag act tca gct  tct gga gct gag gac  ctc agt ggg          4479
```

Glu Gly Leu Glu Thr Ser Ala Ser Gly Ala Glu Asp Leu Ser Gly
     1460   1465   1470

ttg cot tct gga aaa gaa gac ttg gtg ggg tca gct tct gga gac   4524
Leu Pro Ser Gly Lys Glu Asp Leu Val Gly Ser Ala Ser Gly Asp
     1475   1480   1485

ttg gac ttg ggc aaa ctg cct tct gga act cta gga agt ggg caa   4569
Leu Asp Leu Gly Lys Leu Pro Ser Gly Thr Leu Gly Ser Gly Gln
     1490   1495   1500

gct cca gaa aca agt ggt ctt ccc tct gga ttt agt ggt gag tat   4614
Ala Pro Glu Thr Ser Gly Leu Pro Ser Gly Phe Ser Gly Glu Tyr
     1505   1510   1515

tct ggg gtg gac ctt gga agt gga cca ccc tct ggc ctg cct gac   4659
Ser Gly Val Asp Leu Gly Ser Gly Pro Pro Ser Gly Leu Pro Asp
     1520   1525   1530

ttt agt gga ctt cca tct gga ttc cca act gtt tcc cta gtg gat   4704
Phe Ser Gly Leu Pro Ser Gly Phe Pro Thr Val Ser Leu Val Asp
     1535   1540   1545

tct aca ttg gtg gaa gtg gtc aca gcc tcc act gca agt gaa ctg   4749
Ser Thr Leu Val Glu Val Val Thr Ala Ser Thr Ala Ser Glu Leu
     1550   1555   1560

gaa ggg agg gga acc att ggc atc agt ggt gca gga gaa ata tct   4794
Glu Gly Arg Gly Thr Ile Gly Ile Ser Gly Ala Gly Glu Ile Ser
     1565   1570   1575

gga ctg ccc tcc agt gag ctg gac att agt gga aga gct agt gga   4839
Gly Leu Pro Ser Ser Glu Leu Asp Ile Ser Gly Arg Ala Ser Gly
     1580   1585   1590

ctc cct tca gga act gaa ctc agt ggc caa gca tct ggg tct cct   4884

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Leu Pro  Ser Gly Thr Glu Leu  Ser Gly Gln Ala Ser  Gly Ser Pro
    1595                  1600                 1605

gat gtc  agt ggg gaa ata cct  gga ctc ttt ggt gtc  agt gga cag      4929
Asp Val  Ser Gly Glu Ile Pro  Gly Leu Phe Gly Val  Ser Gly Gln
    1610                  1615                 1620

cca tca  ggg ttt cct gac act  agt ggg gaa aca tct  gga gtg act      4974
Pro Ser  Gly Phe Pro Asp Thr  Ser Gly Glu Thr Ser  Gly Val Thr
    1625                  1630                 1635

gag ctt  agc ggg ctg tcc tct  gga caa cca ggt gtt  agt gga gaa      5019
Glu Leu  Ser Gly Leu Ser Ser  Gly Gln Pro Gly Val  Ser Gly Glu
    1640                  1645                 1650

gca tct  gga gtt ctt tat ggc  act agt caa ccc ttt  ggc ata act      5064
Ala Ser  Gly Val Leu Tyr Gly  Thr Ser Gln Pro Phe  Gly Ile Thr
    1655                  1660                 1665

gat ctg  agt gga gaa aca tct  ggg gtc cct gat ctc  agt ggg cag      5109
Asp Leu  Ser Gly Glu Thr Ser  Gly Val Pro Asp Leu  Ser Gly Gln
    1670                  1675                 1680

cct tca  ggg tta cca ggg ttc  agt ggg gca aca tca  gga gtc cct      5154
Pro Ser  Gly Leu Pro Gly Phe  Ser Gly Ala Thr Ser  Gly Val Pro
    1685                  1690                 1695

gac ctg  gtt tct ggt acc acg  agt ggc agc ggt gaa  tct tct ggg      5199
Asp Leu  Val Ser Gly Thr Thr  Ser Gly Ser Gly Glu  Ser Ser Gly
    1700                  1705                 1710

att aca  ttt gtg gac acc agt  ttg gtt gaa gtg gcc  cct act aca      5244
Ile Thr  Phe Val Asp Thr Ser  Leu Val Glu Val Ala  Pro Thr Thr
    1715                  1720                 1725

ttt aaa  gaa gaa gaa ggc tta  ggg tct gtg gaa ctc  agt ggc ctc      5289
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Phe Lys Glu Glu Glu Gly Leu Gly Ser Val Glu Leu Ser Gly Leu
    1730   1735   1740

cct tcc gga gag gca gat ctg tca ggc aaa tct ggg atg gtg gat   5334
Pro Ser Gly Glu Ala Asp Leu Ser Gly Lys Ser Gly Met Val Asp
    1745   1750   1755

gtc agt gga cag ttt tct gga aca gtc gat tcc agt ggg ttt aca   5379
Val Ser Gly Gln Phe Ser Gly Thr Val Asp Ser Ser Gly Phe Thr
    1760   1765   1770

tcc cag act ccg gaa ttc agt ggc cta cca agt ggc ata gct gag   5424
Ser Gln Thr Pro Glu Phe Ser Gly Leu Pro Ser Gly Ile Ala Glu
    1775   1780   1785

gtc agt gga gaa tcc tca aga gct gag att ggg agc agc ctg ccc ,   5469
Val Ser Gly Glu Ser Ser Arg Ala Glu Ile Gly Ser Ser Leu Pro
    1790   1795   1800

tcg gga gca tat tat ggc agt gga act cca tct agt ttc ccc acg   5514
Ser Gly Ala Tyr Tyr Gly Ser Gly Thr Pro Ser Ser Phe Pro Thr
    1805   1810   1815

gtc tct ctt gta gac aga act ttg gtg gaa tct gta acc cag gct   5559
Val Ser Leu Val Asp Arg Thr Leu Val Glu Ser Val Thr Gln Ala
    1820   1825   1830

cca aca gcc caa gag gca gga gaa ggg cct tct ggc att tta gaa   5604
Pro Thr Ala Gln Glu Ala Gly Glu Gly Pro Ser Gly Ile Leu Glu
    1835   1840   1845

ctc agt ggt gct cat tct gga gca cca gac atg tct ggg gag cat   5649
Leu Ser Gly Ala His Ser Gly Ala Pro Asp Met Ser Gly Glu His
    1850   1855   1860

tct gga ttt ctg gac cta agt ggg ctg cag tcc ggg ctg ata gag   5694

Ser Gly Phe Leu Asp Leu Ser Gly Leu Gln Ser Gly Leu Ile Glu
    1865   1870   1875

ccc agc gga gag cca cca ggt act cca tat ttt agt ggg gat ttt   5739
Pro Ser Gly Glu Pro Pro Gly Thr Pro Tyr Phe Ser Gly Asp Phe
    1880   1885   1890

gcc agc acc acc aat gta agt gga gaa tcc tct gta gcc atg agc   5784
Ala Ser Thr Thr Asn Val Ser Gly Glu Ser Ser Val Ala Met Gly
    1895   1900   1905

acc agt gga gag gcc tca gga ctt cca gaa gtt act tta atc act   5829
Thr Ser Gly Glu Ala Ser Gly Leu Pro Glu Val Thr Leu Ile Thr
    1910   1915   1920

tct gag ttc gtg gag ggt gtt act gaa cca act att tct cag gaa . .5874
Ser Glu Phe Val Glu Gly Val Thr Glu Pro Thr Ile Ser Gln Glu
    1925   1930   1935

cta ggc caa agg ccc cct gtg aca cac aca ccc cag ctt ttt gag   5919
Leu Gly Gln Arg Pro Pro Val Thr His Thr Pro Gln Leu Phe Glu
    1940   1945   1950

tcc agt gga aaa gtc tcc aca gct ggg gac att agt gga gct acc   5964
Ser Ser Gly Lys Val Ser Thr Ala Gly Asp Ile Ser Gly Ala Thr
    1955   1960   1965

cca gtg ctc cct ggg tct gga gta gaa gta tca tca gtc cca gaa   6009
Pro Val Leu Pro Gly Ser Gly Val Glu Val Ser Ser Val Pro Glu
    1970   1975   1980

tct agc agt gag aag tcc gcc tat cct gaa gct ggg ttc ggg gca   6054
Ser Ser Ser Glu Thr Ser Ala Tyr Pro Glu Ala Gly Phe Gly Ala
    1985   1990   1995

tct gcc gcc cct gag gcc agc aga gaa gat tct ggg tcc cct gat   6099

Ser Ala Ala Pro Glu Ala Ser Arg Glu Asp Ser Gly Ser Pro Asp
    2000   2005   2010

ctg agt gaa acc acc tct gca ttc cac gas gct aac ctt gag aga   6144
Leu Ser Glu Thr Thr Ser Ala Phe His Glu Ala Asn Leu Glu Arg
    2015   2020   2025

tcc tct agc cta gga gtg agc ggc agc act ttg aca ttt caa gaa   6189
Ser Ser Gly Leu Gly Val Ser Gly Ser Thr Leu Thr Phe Gln Glu
    2030   2035   2040

ggc gag gcg tcc gct gcc cca gaa gtg agt gga gaa tcc acc acc   6234
Gly Glu Ala Ser Ala Ala Pro Glu Val Ser Gly Glu Ser Thr Thr
    2045   2050   2055

acc agt gat gtg gga aca gag gca cca ggc ttg cct tca gcc act   6279
Thr Ser Asp Val Gly Thr Glu Ala Pro Gly Leu Pro Ser Ala Thr
    2060   2065   2070

ccc acg gct tct gga gac agg act gaa atc agc gga gac ctg tct   6324
Pro Thr Ala Ser Gly Asp Arg Thr Glu Ile Ser Gly Asp Leu Ser
    2075   2080   2085

ggt cac acc tcg cag ctg ggc gtt gtc atc agc acc agc atc cca   6369
Gly His Thr Ser Gln Leu Gly Val Val Ile Ser Thr Ser Ile Pro
    2090   2095   2100

gag tct gag tgg acc cag cag acc cag cgc cct gca gag acg cat   6414
Glu Ser Glu Trp Thr Gln Gln Thr Gln Arg Pro Ala Glu Thr His
    2105   2110   2115

cta gaa att gag tcc tca agc ctc ctg tac tca gga gaa gag act   6459
Leu Glu Ile Glu Ser Ser Ser Leu Leu Tyr Ser Gly Glu Glu Thr
    2120   2125   2130

cac aca gtc gaa aca gcc acc tcc cca aca gat gct tcc atc cca   6504

His Thr Val Glu Thr Ala Thr Ser Pro Thr Asp Ala Ser Ile Pro
 2135   2140   2145

gct tct ccg gaa tgg aaa cgt gaa tca gaa tca act gct gca gac 6549
Ala Ser Pro Glu Trp Lys Arg Glu Ser Glu Ser Thr Ala Ala Asp
 2150   2155   2160

cag gag gta tgt gag gag ggc tgg aac aag tac cag ggc cac tgt 6594
Gln Glu Val Cys Glu Glu Gly Trp Asn Lys Tyr Gln Gly His Cys
 2165   2170   2175

tac cgc cac ttc ccg gac cgc gag acc tgg gtg gat gct gag cgc 6639
Tyr Arg His Phe Pro Asp Arg Glu Thr Trp Val Asp Ala Glu Arg
 2180   2185   2190

cgg tgt cgg gag cag cag tca cac ctg agc agc atc gtc acc ccc 6684
Arg Cys Arg Glu Gln Gln Ser His Leu Ser Ser Ile Val Thr Pro
 2195   2200   2205

gag gag cag gag ttt gtc aac aac aat gcc caa gac tac cag tgg 6729
Glu Glu Gln Glu Phe Val Asn Asn Asn Ala Gln Asp Tyr Gln Trp
 2210   2215   2220

atc ggc ctg aac gac agg acc atc gaa ggg gac ttc cgc tgg tca 6774
Ile Gly Leu Asn Asp Arg Thr Ile Glu Gly Asp Phe Arg Trp Ser
 2225   2230   2235

gat gga cac ccc atg caa ttt gag aac tgg cgc ccc aac cag cct 6819
Asp Gly His Pro Met Gln Phe Glu Asn Trp Arg Pro Asn Gln Pro
 2240   2245   2250

gac aac ttt ttt gcc gct gga gag gcc tgt gtg gtg atg atc tgg 6864
Asp Asn Phe Phe Ala Ala Gly Glu Asp Cys Val Val Met Ile Trp
 2255   2260   2265

cac gag aag ggc gag tgg aat gat gtt ccc tgc aat tac cac gtc 6909

His Glu Lys Gly Glu Trp Asn Asp Val Pro Cys Asn Tyr His Leu
     2270   2275   2280

ccc ttc acg tgt aaa aag ggc aca gcc acc acc tac aaa cgc aga   6954
Pro Phe Thr Cys Lys Lys Gly Thr Ala Thr Thr Tyr Lys Arg Arg
     2285   2290   2295

cta cag aag cgg agc tca cgg cac cct cgg agg agc cgc ccc agc   6999
Leu Gln Lys Arg Ser Ser Arg His Pro Arg Arg Ser Arg Pro Ser
     2300   2305   2310

aca gcc cac tga gaagagcttc caggacgcac ccaggacgct gagcccagga   7051
Thr Ala His
     2315

gcctgccagg ctgacgtgca tcccacccag acggtgtcct cttcttgtcg ctttttgtca 7111

tstaaggaat cccattaaaa aaaeaa   7137

<210> 20
<211> 2316
<212> PRT
<213> Homo sapiens

<400> 20

Met Thr Thr Leu Leu Trp Val Phe Val Thr Leu Arg Val Ile Thr Ala
1   5   10   15

Ala Val Thr Val Glu Thr Ser Asp His Asp Asn Ser Leu Ser Val Ser
          20   25   30

Ile Pro Gln Pro Ser Pro Leu Arg Val Leu Leu Gly Thr Ser Leu Thr

                35   40   45

Ile Pro Cys Tyr Phe Ile Asp Pro Met His Pro Val Thr Thr Ala Pro
      50   55   60

Ser Thr Ala Pro Leu Ala Pro Arg Ile Lys Trp Ser Arg Val Ser Lys
65   70   75   80

Glu Lys Glu Val Val Leu Leu Val Ala Thr Glu Gly Arg Val Arg Val
                85   90   95

Asn Ser Ala Tyr Gln Asp Lys Val Ser Leu Pro Asn Tyr Pro Ala Ile
            100   105   110

Pro Ser Asp Ala Thr Leu Glu Val Gln Ser Leu Arg Ser Asn Asp Ser
            115   120   125

Gly Val Tyr Arg Cys Glu Val Met His Gly Ile Glu Asp Ser Glu Ala
      130   135   140

Thr Leu Glu Val Val Val Lys Gly Ile Val Phe His Tyr Arg Ala Ile
145   150   155   160

Ser Thr Arg Tyr Thr Leu Asp Phe Asp Arg Ala Gln Arg Ala Cys Leu
                165   170   175

Gln Asn Ser Ala Ile Ile Ala Thr Pro Glu Gln Leu Gln Ala Ala Tyr

                180   185   190

Glu Asp Gly Phe His Gln Cys Asp Ala Gly Trp Leu Ala Asp Gln Thr
        195   200   205

Val Arg Tyr Pro Ile His Thr Pro Arg Glu Gly Cys Tyr Gly Asp Lys
    210   215   220

Asp Glu Phe Pro Gly Val Arg Thr Tyr Gly Ile Arg Asp Thr Asn Glu
225   230   235   240

Thr Tyr Asp Val Tyr Cys Phe Ala Glu Glu Met Glu Gly Glu Val Phe
                245   250   255

Tyr Ala Thr Ser Pro Glu Lys Phe Thr Phe Gln Glu Ala Ala Asn Glu
            260   265   270

Cys Arg Arg Leu Gly Ala Arg Leu Ala Thr Thr Gly His Val Tyr Leu
        275   280   285

Ala Trp Gln Ala Gly Met Asp Met Cys Ser Ala Gly Trp Leu Ala Asp
    290   295   300

Arg Ser Val Arg Tyr Pro Ile Ser Lys Ala Arg Pro Asn Cys Gly Gly
305   310   315   320

Asn Leu Leu Gly Val Arg Thr Val Tyr Val His Ala Asn Gln Thr Gly

                        325   330   335

Tyr Pro Asp Pro Ser Ser Arg Tyr Asp Ala Ile Cys Tyr Thr Gly Glu
            340   345   350

Asp Phe Val Asp Ile Pro Glu Asn Phe Phe Gly Val Gly Gly Glu Glu
        355   360   365

Asp Ile Thr Val Gln Thr Val Thr Trp Pro Asp Met Glu Leu Pro Leu
    370   375   380

Pro Arg Asn Ile Thr Glu Gly Glu Ala Arg Gly Ser Val Ile Leu Thr
385   390   395   400

Val Lys Pro Ile Phe Glu Val Ser Pro Ser Pro Leu Glu Pro Glu Glu
            405   410   415

Pro Phe Thr Phe Ala Pro Glu Ile Gly Ala Thr Ala Phe Ala Glu Val
            420   425   430

Glu Asn Glu Thr Gly Glu Ala Thr Arg Pro Trp Gly Phe Pro Thr Pro
        435   440   445

Gly Leu Gly Pro Ala Thr Ala Phe Thr Ser Glu Asp Leu Val Val Gln
    450   455   460

Val Thr Ala Val Pro Gly Gln Pro His Leu Pro Gly Gly Val Val Phe

465   470   475   480

His Tyr Arg Pro Gly Pro Thr Arg Tyr Ser Leu Thr Phe Glu Glu Ala
                485   490   495

Gln Gln Ala Cys Pro Gly Thr Gly Ala Val Ile Ala Ser Pro Glu Gln
                500   505   510

Leu Gln Ala Ala Tyr Glu Ala Gly Tyr Glu Gln Cys Asp Ala Gly Trp
        515   520   525

Leu Arg Asp Gln Thr Val Arg Tyr Pro Ile Val Ser Pro Arg Thr Pro
    530   535   540

Cys Val Gly Asp Lys Asp Ser Ser Pro Gly Val Arg Thr Tyr Gly Val
545   550   555   560

Arg Pro Ser Thr Glu Thr Tyr Asp Val Tyr Cys Phe Val Asp Arg Leu
                565   570   575

Glu Gly Glu Val Phe Phe Ala Thr Arg Leu Glu Gln Phe Thr Phe Gln
                580   585   590

Glu Ala Leu Glu Phe Cys Glu Ser His Asn Ala Thr Ala Thr Thr Gly
        595   600   605

Gln Leu Tyr Ala Ala Trp Ser Arg Gly Leu Asp Lys Cys Tyr Ala Gly

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            610                    615                    620


Trp Leu Ala Asp Gly Ser Leu Arg Tyr Pro Ile Val Thr Pro Arg Pro
625                    630                    635                    640


Ala Cys Gly Gly Asp Lys Pro Gly Val Arg Thr Val Tyr Leu Tyr Pro
                  645                    650                    655


Asn Gln Thr Gly Leu Pro Asp Pro Leu Ser Arg His His Ala Phe Cys
                  660                    665                    670


Phe Arg Gly Ile Ser Ala Val Pro Ser Pro Gly Glu Glu Glu Gly Gly
            675                    680                    685


Thr Pro Thr Ser Pro Ser Gly Val Glu Glu Trp Ile Val Thr Gln Val
            690                    695                    700


Val Pro Gly Val Ala Ala Val Pro Val Glu Glu Glu Thr Thr Ala Val
705                    710                    715                    720


Pro Ser Gly Glu Thr Thr Ala Ile Leu Glu Phe Thr Thr Glu Pro Glu
                  725                    730                    735


Asn Gln Thr Glu Trp Glu Pro Ala Tyr Thr Pro Val Gly Thr Ser Pro
                  740                    745                    750


Leu Pro Gly Ile Leu Pro Thr Trp Pro Pro Thr Gly Ala Glu Thr Glu
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755   760   765

Glu Ser Thr Glu Gly Pro Ser Ala Thr Glu Val Pro Ser Ala Ser Glu
   770   775   780

Glu Pro Ser Pro Ser Glu Val Pro Phe Pro Ser Glu Glu Pro Ser Pro
785   790   795   800

Ser Glu Glu Pro Phe Pro Ser Val Arg Pro Phe Pro Ser Val Glu Leu
             805   810   815

Phe Pro Ser Glu Glu Pro Phe Pro Ser Lys Glu Pro Ser Pro Ser Glu
             820   825   830

Glu Pro Ser Ala Ser Glu Glu Pro Tyr Thr Pro Ser Pro Pro Glu Pro
      835   840   845

Ser Trp Thr Glu Leu Pro Ser Ser Gly Glu Glu Ser Gly Ala Pro Asp
   850   855   860

Val Ser Gly Asp Phe Thr Gly Ser Gly Asp Val Ser Gly His Leu Asp
865   870   875   880

Phe Ser Gly Gln Leu Ser Gly Asp Arg Ala Ser Gly Leu Pro Ser Gly
             885   890   895

Asp Leu Asp Ser Ser Gly Leu Thr Ser Thr Val Gly Ser Gly Leu Thr

                    900   905   910

Val Glu Ser Gly Leu Pro Ser Gly Asp Glu Glu Arg Ile Glu Trp Pro
          915   920   925

Ser Thr Pro Thr Val Gly Glu Leu Pro Ser Gly Ala Glu Ile Leu Glu
     930   935   940

Gly Ser Ala Ser Gly Val Gly Asp Leu Ser Gly Leu Pro Ser Gly Glu
945   950   955   960

Val Leu Glu Thr Ser Ala Ser Gly Val Gly Asp Leu Ser Gly Leu Pro
               965   970   975

Ser Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu Asp Ile Ser
               980   985   990

Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu
          995   1000   1005

Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala
     1010   1015   1020

Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu
     1025   1030   1035

Glu Thr Thr Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser

1040   1045   1050

Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu Asp Ile Ser
    1055   1060   1065

Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly Val
    1070   1075   1080

Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala
    1085   1090   1095

Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val
    1100   1105   1110

Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro
    1115   1120   1125

Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile
    1130   1135   1140

Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly
    1145   1150   1155

Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr
    1160   1165   1170

Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu

1175   1180   1185

Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu
    1190   1195   1200

Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp
    1205   1210   1215

Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro
    1220   1225   1230

Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu
    1235   1240   1245

Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly
    1250   1255   1260

Glu Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly
    1265   1270   1275

Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu
    1280   1285   1290

Glu Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala
    1295   1300   1305

Pro Gly Val Asp Glu Ile Ser Gly Leu Pro Ser Gly Glu Val Leu

1310 1315 1320

Glu Thr Thr Ala Pro Gly Val Glu Glu Ile Ser Gly Leu Pro Ser
    1325   1330   1335

Gly Glu Val Leu Glu Thr Ser Thr Ser Ala Val Gly Asp Leu Ser
    1340   1345   1350

Gly Leu Pro Ser Gly Gly Glu Val Leu Glu Ile Ser Val Ser Gly
    1355   1360   1365

Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Val Glu Thr
    1370   1375   1380

Ser Ala Ser Gly Ile Glu Asp Val Ser Glu Leu Pro Ser Gly Glu
    1385   1390   1395

Gly Leu Glu Thr Ser Ala Ser Gly Val Glu Asp Leu Ser Arg Leu
    1400   1405   1410

Pro Ser Gly Glu Glu Val Leu Glu Ile Ser Ala Ser Gly Phe Gly
    1415   1420   1425

Asp Leu Ser Gly Val Pro Ser Gly Gly Glu Gly Leu Glu Thr Ser
    1430   1435   1440

Ala Ser Glu Val Gly Thr Asp Leu Ser Gly Leu Pro Ser Gly Arg

1445   1450   1455

Glu Gly Leu Glu Thr Ser Ala Ser Gly Ala Glu Asp Leu Ser Gly
   1460   1465   1470

Leu Pro Ser Gly Lys Glu Asp Leu Val Gly Ser Ala Ser Gly Asp
   1475   1480   1485

Leu Asp Leu Gly Lys Leu Pro Ser Gly Thr Leu Gly Ser Gly Gln
   1490   1495   1500

Ala Pro Glu Thr Ser Gly Leu Pro Ser Gly Phe Ser Gly Glu Tyr
   1505   1510   1515

Ser Gly Val Asp Leu Gly Ser Gly Pro Pro Ser Gly Leu Pro Asp
   1520   1525   1530

Phe Ser Gly Leu Pro Ser Gly Phe Pro Thr Val Ser Leu Val Asp
   1535   1540   1545

Ser Thr Leu Val Glu Val Val Thr Ala Ser Thr Ala Ser Glu Leu
   1550   1555   1560

Glu Gly Arg Gly Thr Ile Gly Ile Ser Gly Ala Gly Glu Ile Ser
   1565   1570   1575

Gly Leu Pro Ser Ser Glu Leu Asp Ile Ser Gly Arg Ala Ser Gly

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        1580                    1585                    1590


Leu Pro  Ser Gly Thr Glu Leu  Ser Gly Gln Ala Ser  Gly Ser Pro
     1595                    1600                    1605


Asp Val  Ser Gly Glu Ile Pro  Gly Leu Phe Gly Val  Ser Gly Gln
     1610                    1615                    1620


Pro Ser  Gly Phe Pro Asp Thr  Ser Gly Glu Thr Ser  Gly Val Thr
     1625                    1630                    1635


Glu Leu  Ser Gly Leu Ser Ser  Gly Gln Pro Gly Val  Ser Gly Glu
     1640                    1645                    1650


Ala Ser  Gly Val Leu Tyr Gly  Thr Ser Gln Pro Phe  Gly Ile Thr
     1655                    1660                    1665


Asp Leu  Ser Gly Glu Thr Ser  Gly Val Pro Asp Leu  Ser Gly Gln
     1670                    1675                    1680


Pro Ser  Gly Leu Pro Gly Phe  Ser Gly Ala Thr Ser  Gly Val Pro
     1685                    1690                    1695


Asp Leu  Val Ser Gly Thr Thr  Ser Gly Ser Gly Glu  Ser Ser Gly
     1700                    1705                    1710


Ile Thr  Phe Val Asp Thr Ser  Leu Val Glu Val Ala  Pro Thr Thr
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1715   1720   1725

Phe Lys Glu Gln Glu Gly Leu Gly Ser Val Glu Leu Ser Gly Leu
    1730   1735   1740

Pro Ser Gly Glu Ala Asp Leu Ser Gly Lys Ser Gly Met Val Asp
    1745   1750   1755

Val Ser Gly Gln Phe Ser Gly Thr Val Asp Ser Ser Gly Phe Thr
    1760   1765   1770

Ser Gln Thr Pro Glu Phe Ser Gly Leu Pro Ser Gly Ile Ala Glu
    1775   1780   1785

Val Ser Gly Glu Ser Ser Arg Ala Glu Ile Gly Ser Ser Leu Pro
    1790   1795   1800

Ser Gly Ala Tyr Tyr Gly Ser Gly Thr Pro Ser Ser Phe Pro Thr
    1805   1810   1815

Val Ser Leu Val Asp Arg Thr Leu Val Glu Ser Val Thr Gln Ala
    1820   1825   1830

Pro Thr Ala Gln Glu Ala Gly Glu Gly Pro Ser Gly Ile Leu Glu
    1835   1840   1845

Leu Ser Gly Ala His Ser Gly Ala Pro Asp Met Ser Gly Glu His

1850   1855   1860

Ser Gly Phe Leu Asp Leu Ser Gly Leu Gln Ser Gly Leu Ile Glu
    1865   1870   1875

Pro Ser Gly Glu Pro Pro Gly Thr Pro Tyr Phe Ser Gly Asp Phe
    1880   1885   1890

Ala Ser Thr Thr Asn Val Ser Gly Glu Ser Ser Val Ala Met Gly
    1895   1900   1905

Thr Ser Gly Glu Ala Ser Gly Leu Pro Glu Val Thr Leu Ile Thr
    1910   1915   1920

Ser Glu Phe Val Glu Gly Val Thr Glu Pro Thr Ile Ser Gln Glu
    1925   1930   1935

Leu Gly Gln Arg Pro Pro Val Thr His Thr Pro Gln Leu Phe Glu
    1940   1945   1950

Ser Ser Gly Lys Val Ser Thr Ala Gly Asp Ile Ser Gly Ala Thr
    1955   1960   1965

Pro Val Leu Pro Gly Ser Gly Val Glu Val Ser Ser Val Pro Glu
    1970   1975   1980

Ser Ser Ser Glu Thr Ser Ala Tyr Pro Glu Ala Gly Phe Gly Ala

          1985   1990   1995

Ser Ala Ala Pro Glu Ala Ser Arg Glu Asp Ser Gly Ser Pro Asp
     2000   2005   2010

Leu Ser Glu Thr Thr Ser Ala Phe His Glu Ala Asn Leu Glu Arg
     2015   2020   2025

Ser Ser Gly Leu Gly Val Ser Gly Ser Thr Leu Thr Phe Gln Glu
     2030   2035   2040

Gly Glu Ala Ser Ala Ala Pro Glu Val Ser Gly Glu Ser Thr Thr
     2045   2050   2055

Thr Ser Asp Val Gly Thr Glu Ala Pro Gly Leu Pro Ser Ala Thr
     2060   2065   2070

Pro Thr Ala Ser Gly Asp Arg Thr Glu Ile Ser Gly Asp Leu Ser
     2075   2080   2085

Gly His Thr Ser Gln Leu Gly Val Val Ile Ser Thr Ser Ile Pro
     2090   2095   2100

Glu Ser Glu Trp Thr Gln Gln Thr Gln Arg Pro Ala Glu Thr His
     2105   2110   2115

Leu Glu Ile Glu Ser Ser Ser Leu Leu Tyr Ser Gly Glu Glu Thr

          2120   2125   2130

His Thr Val Glu Thr Ala Thr Ser Pro Thr Asp Ala Ser Ile Pro
     2135   2140   2145

Ala Ser Pro Glu Trp Lys Arg Glu Ser Glu Ser Thr Ala Ala Asp
     2150   2155   2160

Gln Glu Val Cys Glu Glu Gly Trp Asn Lys Tyr Gln Gly His Cys
     2165   2170   2175

Tyr Arg His Phe Pro Asp Arg Glu Thr Trp Val Asp Ala Glu Arg
     2180   2185   2190

Arg Cys Arg Glu Gln Gln Ser His Leu Ser Ser Ile Val Thr Pro
     2195   2200   2205

Glu Glu Gln Glu Phe Val Asn Asn Asn Ala Gln Asp Tyr Gln Trp
     2210   2215   2220

Ile Gly Leu Asn Asp Arg Thr Ile Glu Gly Asp Phe Arg Trp Ser
     2225   2230   2235

Asp Gly His Pro Met Gln Phe Glu Asn Trp Arg Pro Asn Gln Pro
     2240   2245   2250

Asp Asn Phe Phe Ala Ala Gly Glu Asp Cys Val Val Met Ile Trp

```
       2255                  2260                  2265


  His Glu  Lys Gly Glu Trp Asn  Asp Val Pro Cys Asn  Tyr His Leu
       2270                  2275                  2280


  Pro Phe  Thr Cys Lys Lys Gly  Thr Ala Thr Thr Tyr  Lys Arg Arg
       2285                  2290                  2295


  Leu Gln  Lys Arg Ser Ser Arg  His Pro Arg Arg Ser  Arg Pro Ser
       2300                  2305                  2310


  Thr Ala  His
       2315


<210>  21
<211>  1108
<212>  DNA
<213>  Homo sapiens


<220>
<221>  CDS
<222>  (143)..(1096)


<400>  21
gagtgagtga gagggcagag gaaatactca atctgtgcca ctcactgcct tgagcctgct      60

tcctcactcc aggactgcca gaggctcact cacttgagcc tgcttcctca ctccaggact     120

gccagaggaa gcaatcacca aa atg aag act gct tta att ttg ctc agc att      172
                         Met Lys Thr Ala Leu Ile Leu Leu Ser Ile
```

                              1   5   10

ttg gga atg gcc tgt gct ttc tca atg aaa aat ttg cat cga aga gtc   220
Leu Gly Met Ala Cys Ala Phe Ser Met Lys Asn Leu His Arg Arg Val
                    15   20   25

aaa ata gag gat tct gaa gaa aat ggg gtc ttt aag tac agg cca cga   268
Lys Ile Glu Asp Ser Glu Glu Asn Gly Val Phe Lys Tyr Arg Pro Arg
               30   35   40

tat tat ctt tac aag cat gcc tac ttt tat cct cat tta aag cga ttt   316
Tyr Tyr Leu Tyr Lys His Ala Tyr Phe Tyr Pro His Leu Lys Arg Phe
          45   50   55

cca gtt cag ggc agt agt gac tca tcc gaa gaa aat gga gat gac agt   364
Pro Val Gln Gly Ser Ser Asp Ser Ser Glu Glu Asn Gly Asp Asp Ser
     60   65   70

tca gaa gag gag gag gaa gaa gag gag act tca aat gaa gga gaa aac   412
Ser Glu Glu Glu Glu Glu Glu Glu Glu Thr Ser Asn Glu Gly Glu Asn
75   80   85   90

aat gaa gaa tcg aat gaa gat gaa gac tct gag gct gag aat acc aca   460
Asn Glu Glu Ser Asn Glu Asp Glu Asp Ser Glu Ala Glu Asn Thr Thr
               95   100   105

ctt tct gct aca aca ctg ggc tat gga gag gac gcc acg cct ggc aca   508
Leu Ser Ala Thr Thr Leu Gly Tyr Gly Glu Asp Ala Thr Pro Gly Thr
          110   115   120

ggg tat aca ggg tta gct gca atc cag ctt ccc aag aag gct ggg gat   556
Gly Tyr Thr Gly Leu Ala Ala Ile Gln Leu Pro Lys Lys Ala Gly Asp
     125   130   135

ata aca aac aaa gct aca aaa gag aag gaa agt gct gaa gaa gaa gag   604
Ile Thr Asn Lys Ala Thr Lys Glu Lys Glu Ser Asp Glu Glu Glu Glu

```
          140                  145                  150

gag gaa gag gaa gga aat gaa aac gaa gaa agc gaa gca gaa gtg gat      652
Glu Glu Glu Glu Gly Asn Glu Asn Glu Glu Ser Glu Ala Glu Val Asp
155                  160                  165                  170


gaa aac gaa caa ggc ata aac ggc acc agt acc aac agc aca gag gca      700
Glu Asn Glu Gln Gly Ile Asn Gly Thr Ser Thr Asn Ser Thr Glu Ala
                    175                  180                  185


gaa aac ggc aac ggc agc agc gga gga gac aat gga gaa gaa ggg gaa      748
Glu Asn Gly Asn Gly Ser Ser Gly Gly Asp Asn Gly Glu Glu Gly Glu
                190                  195                  200


gaa gaa agt gtc act gga gcc aat gca gaa ggc acc aca gag acc gga      796
Glu Glu Ser Val Thr Gly Ala Asn Ala Glu Gly Thr Thr Glu Thr Gly
            205                  210                  215


ggg cag ggc aag ggc acc tcg aag aca aca acc tct cca aat ggt ggg      844
Gly Gln Gly Lys Gly Thr Ser Lys Thr Thr Thr Ser Pro Asn Gly Gly
    220                  225                  230


ttt gaa cct aca acc cca caa gtc tat aga acc act tcc cca cct          892
Phe Glu Pro Thr Thr Pro Pro Gln Val Tyr Arg Thr Thr Ser Pro Pro
235                  240                  245                  250


ttt ggg aaa acc acc acc gtt gaa tac gag ggg gag tac gaa tac acg      940
Phe Gly Lys Thr Thr Thr Val Glu Tyr Glu Gly Glu Tyr Glu Tyr Thr
                255                  260                  265


ggc gtc aat gaa tac gac aat gga tat gaa atc tat gaa agt gag aac      988
Gly Val Asn Glu Tyr Asp Asn Gly Tyr Glu Ile Tyr Glu Ser Glu Asn
                270                  275                  280


ggg gaa cct cgt ggg gac aat tac cga gcc tat gaa gat gag tac agc     1036
Gly Glu Pro Arg Gly Asp Asn Tyr Arg Ala Tyr Glu Asp Glu Tyr Ser
```

        285   290   295

tac ttt aaa gga caa ggc tac gat ggc tat gat ggt cag aat tac tac   1084
Tyr Phe Lys Gly Gln Gly Tyr Asp Gly Tyr Asp Gly Gln Asn Tyr Tyr
    300   305   310

cac cac cag tga agctccagcc tg   1108
His His Gln
315

<210> 22
<211> 317
<212> PRT
<213> Homo sapiens

<400> 22

Met Lys Thr Ala Leu Ile Leu Leu Ser Ile Leu Gly Met Ala Cys Ala
1   5   10   15

Phe Ser Met Lys Asn Leu His Arg Arg Val Lys Ile Glu Asp Ser Glu
            20   25   30

Glu Asn Gly Val Phe Lys Tyr Arg Pro Arg Tyr Tyr Leu Tyr Lys His
        35   40   45

Ala Tyr Phe Tyr Pro His Leu Lys Arg Phe Pro Val Gln Gly Ser Ser
    50   55   60

Asp Ser Ser Glu Glu Asn Gly Asp Asp Ser Ser Glu Glu Glu Glu Glu
65   70   75   80

Glu Glu Glu Thr Ser Asn Glu Gly Glu Asn Asn Glu Glu Ser Asn Glu
                85   90   95

Asp Glu Asp Ser Glu Ala Glu Asn Thr Thr Leu Ser Ala Thr Thr Leu
               100   105   110

Gly Tyr Gly Glu Asp Ala Thr Pro Gly Thr Gly Tyr Thr Gly Leu Ala
              115   120   125

Ala Ile Gln Leu Pro Lys Lys Ala Gly Asp Ile Thr Asn Lys Ala Thr
          130   135   140

Lys Glu Lys Glu Ser Asp Glu Glu Glu Glu Glu Glu Glu Glu Gly Asn
145   150   155   160

Glu Asn Glu Glu Ser Glu Ala Glu Val Asp Glu Asn Glu Gln Gly Ile
               165   170   175

Asn Gly Thr Ser Thr Asn Ser Thr Glu Ala Glu Asn Gly Asn Gly Ser
              180   185   190

Ser Gly Gly Asp Asn Gly Glu Glu Gly Glu Glu Glu Ser Val Thr Gly
          195   200   205

Ala Asn Ala Glu Gly Thr Thr Glu Thr Gly Gly Gln Gly Lys Gly Thr
          210   215   220

Ser Lys Thr Thr Thr Ser Pro Asn Gly Gly Phe Glu Pro Thr Thr Pro
225   230   235   240

Pro Gln Val Tyr Arg Thr Thr Ser Pro Pro Phe Gly Lys Thr Thr Thr
                245   250   255

Val Glu Tyr Glu Gly Glu Tyr Glu Tyr Thr Gly Val Asn Glu Tyr Asp
            260   265   270

Asn Gly Tyr Glu Ile Tyr Glu Ser Glu Asn Gly Glu Pro Arg Gly Asp
            275   280   285

Asn Tyr Arg Ala Tyr Glu Asp Glu Tyr Ser Tyr Phe Lys Gly Gln Gly
        290   295   300

Tyr Asp Gly Tyr Asp Gly Gln Asn Tyr Tyr His His Gln
305   310   315

<210> 23
<211> 498
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (19)..(321)

<400> 23

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sgcagccacc gagacacc atg aga gcc ctc aca ctc ctc gcc cta ttg gca        51
                    Met Arg Ala Leu Thr Leu Leu Ala Leu Leu Ala
                     1           5                  10

ctg gcc gca ctt tgc atc gct agc cag gca ggt gcg aag ccc agc ggt        99
Leu Ala Ala Leu Cys Ile Ala Gly Gln Ala Gly Ala Lys Pro Ser Gly
           15              20                  25

gca gag tcc agc aaa ggt gca gcc ttt gtg tcc aag cag gag ggc agc       147
Ala Glu Ser Ser Lys Gly Ala Ala Phe Val Ser Lys Gln Glu Gly Ser
         30              35                  40

gag gta gtg aag aga ccc agg cgc tac ctg tat caa tgg ctg gga gcc       195
Glu Val Val Lys Arg Pro Arg Arg Tyr Leu Tyr Gln Trp Leu Gly Ala
       45              50                  55

cca gtc ccc tac ccg gat ccc ctg gag ccc agg agg gag gtg tgt gag       243
Pro Val Pro Tyr Pro Asp Pro Leu Glu Pro Arg Arg Glu Val Cys Glu
60              65                  70                  75

ctc aat ccg gac tgt gac gag ttg gct gac cac atc ggc ttt cag gag       291
Leu Asn Pro Asp Cys Asp Glu Leu Ala Asp His Ile Gly Phe Gln Glu
             80                  85                  90

gcc tat cgg cgc ttc tac ggc ccg gtc tag ggtgtcgtc tgctggcctg          341
Ala Tyr Arg Arg Phe Tyr Gly Pro Val
             95                  100

gccggcaacc ccagttctgc toctctccag gcacccttct ttcctcttcc ccttgccctt      401

gccctgacct cccagcccta tggatgtggg gtccccatcc tcccagcctgc tcccaaataa      461

actccagaag aggaatctga aaaaaaaaa aaaaaa                                 498
```

<210> 24
<211> 100
<212> PRT
<213> Homo sapiens

<400> 24

Met Arg Ala Leu Thr Leu Leu Ala Leu Leu Ala Leu Ala Ala Leu Cys
1   5   10   15

Ile Ala Gly Gln Ala Gly Ala Lys Pro Ser Gly Ala Glu Ser Ser Lys
                20   25   30

Gly Ala Ala Phe Val Ser Lys Gln Glu Gly Ser Glu Val Val Lys Arg
            35   40   45

Pro Arg Arg Tyr Leu Tyr Gln Trp Leu Gly Ala Pro Val Pro Tyr Pro
        50   55   60

Asp Pro Leu Glu Pro Arg Arg Glu Val Cys Glu Leu Asn Pro Asp Cys
65   70   75   80

Asp Glu Leu Ala Asp His Ile Gly Phe Gln Glu Ala Tyr Arg Arg Phe
                85   90   95

Tyr Gly Pro Val
            100

<210> 25

<211> 2383
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (320)..(1825)

<400> 25

ctccttcaag ccctcagtca gttgtgcagg agaaaggggg cggttggctt totcctttca   60

agaacgagtt attttcagct gctgactgga gacggtgcac gtctggatac gagagcaatt   120

ccactatggg actggatgca aacacacacc cggcagactt caagagtctc agactgaggg   180

gaaagccttt ccttctgctg ctactgctgc tccgcctgct tttgaaagtc cactcctttc   240

atggtttttc ctgccaaaac agaggcaacc ttgctgctgc cgctgttctc tttggtgtca   300

ttcagcggct ggccagagg atg aga ctc ccc aaa ctc ctc act ttc ttg ctt   352
                             Met Arg Leu Pro Lys Leu Leu Thr Phe Leu Leu
                             1   5   10

tgg tac ctg gct tgg ctg gac ctg gaa ttc atc tgc act gtg ttg ggt   400
Trp Tyr Leu Ala Trp Leu Asp Leu Glu Phe Ile Cys Thr Val Leu Gly
            15   20   25

ggc cct gac ttg ggc cag aga ccc cag ggg acc agg cca gga ttg gcc   448
Ala Pro Asp Leu Gly Gln Arg Pro Gln Gly Thr Arg Pro Gly Leu Ala
        30   35   40

aaa gca gag gcc aag gag agg ccc ccc ctg gcc cgg aac gtc ttc agg   496
Lys Ala Glu Ala Lys Glu Arg Pro Pro Leu Ala Arg Asn Val Phe Arg
  45   50   55

```
cca ggg ggt cac agc tat agt ggg ggg gcc acc aat gcc aat gcc agg      544
Pro Gly Gly His Ser Tyr Ser Gly Gly Gly Ala Thr Asn Ala Asn Ala Arg
60              65              70              75

gca aag gga ggc acc ggg cag aca gga ggc ctg aca cag ccc aag aag      592
Ala Lys Gly Gly Thr Gly Gln Thr Gly Gly Leu Thr Gln Pro Lys Lys
                80              85              90

gat gaa ccc aaa aag ctg ccc ccc aga ccg ggc ggc cct gaa ccc aag      640
Asp Glu Pro Lys Lys Leu Pro Pro Arg Pro Gly Gly Pro Glu Pro Lys
                95              100             105

cca gga cac cct ccc caa aca agg cag gct aca gcc cgg act gtg acc      688
Pro Gly His Pro Pro Gln Thr Arg Gln Ala Thr Ala Arg Thr Val Thr
        110             115             120

cca aaa gga cag ctt ccc gga ggc aag gca ccc cca aaa gca gga tct      736
Pro Lys Gly Gln Leu Pro Gly Gly Lys Ala Pro Pro Lys Ala Gly Ser
    125             130             135

gtc ccc agc tcc ttc ctg ctg aag aag gcc agg gag ccc ggg ccc cca      784
Val Pro Ser Ser Phe Leu Leu Lys Lys Ala Arg Glu Pro Gly Pro Pro
140             145             150             155

cga gag ccc aag gag ccg ttt cgc cca ccc ccc atc aca ccc cac gag      832
Arg Glu Pro Lys Glu Pro Phe Arg Pro Pro Pro Ile Thr Pro His Glu
                160             165             170

tac atg ctc tcg ctg tac agg acg ctg tcc gat gct gac aga aag gga      880
Tyr Met Leu Ser Leu Tyr Arg Thr Leu Ser Asp Ala Asp Arg Lys Gly
            175             180             185

ggc aac agc agc gtg aag ttg gag gct ggc ctg gcc aac acc atc acc      928
Gly Asn Ser Ser Val Lys Leu Glu Ala Gly Leu Ala Asn Thr Ile Thr
        190             195             200
```

```
agc ttt att gac aaa gag caa gat gac cga ggt ccc gtg gtc agg aag    976
Ser Phe Ile Asp Lys Gly Gln Asp Asp Arg Gly Pro Val Val Arg Lys
    205                 210                 215

cag agg tac gtg ttt gac att agt gcc ctg gag aag gat ggg ctg ctg    1024
Gln Arg Tyr Val Phe Asp Ile Ser Ala Leu Glu Lys Asp Gly Leu Leu
220                 225                 230                 235

ggg gcc gag ctg cgg atc ttg cgg aag aag ccc tcg gac acg gcc aag    1072
Gly Ala Glu Leu Arg Ile Leu Arg Lys Lys Pro Ser Asp Thr Ala Lys
                240                 245                 250

cca gcg gcc ccc gga ggc ggg cgg gct gcc cag ctg aag ctg tcc agc    1120
Pro Ala Ala Pro Gly Gly Gly Arg Ala Ala Gln Leu Lys Leu Ser Ser
                255                 260                 265

tgc ccc agc ggc cgg cag ccg gcc tcc ttg ctg gat gtg cgc tcc gtg    1168
Cys Pro Ser Gly Arg Gln Pro Ala Ser Leu Leu Asp Val Arg Ser Val
        270                 275                 280

cca ggc ctg gac gga tct ggc tgg gag gtg ttc gac atc tgg aag ctc    1216
Pro Gly Leu Asp Gly Ser Gly Trp Glu Val Phe Asp Ile Trp Lys Leu
    285                 290                 295

ttc cga aac ttt aag aac tcg gcc cag ctg tgc ctg gag ctg gag gcc    1264
Phe Arg Asn Phe Lys Asn Ser Ala Gln Leu Cys Leu Glu Leu Glu Ala
300                 305                 310                 315

tgg gaa cgg ggc agg gcc gtg gac ctc cgt ggc ctg ggc ttc gac cgc    1312
Trp Glu Arg Gly Arg Ala Val Asp Leu Arg Gly Leu Gly Phe Asp Arg
                320                 325                 330

gcc gcc cgg cag gtc cac gag aag gcc ctg ttc ctg gtg ttt ggc cgc    1360
Ala Ala Arg Gln Val His Glu Lys Ala Leu Phe Leu Val Phe Gly Arg
    335                 340                 345
```

```
acc aag aaa cgg gac ctg ttc ttt aat gag att aag gcc cgc tct ggc    1408
Thr Lys Lys Arg Asp Leu Phe Phe Asn Glu Ile Lys Ala Arg Ser Gly
        350                 355                 360

cag gac gat aag acc gtg tat gag tac ctg ttc agc cag cgg cga aaa    1456
Gln Asp Asp Lys Thr Val Tyr Glu Tyr Leu Phe Ser Gln Arg Arg Lys
        365                 370                 375

cgg cgg gcc ccg ctg gcc act cgc cag ggc aag cga ccc agc aag aac    1504
Arg Arg Ala Pro Leu Ala Thr Arg Gln Gly Lys Arg Pro Ser Lys Asn
380                 385                 390                 395

ctt aag gct cgc tgc agt cgg aag gca ctg cat gtc aac ttc aag gac    1552
Leu Lys Ala Arg Cys Ser Arg Lys Ala Leu His Val Asn Phe Lys Asp
                    400                 405                 410

atg ggc tgg gac gac tgg atc atc gca ccc ctt gag tac gag gct ttc    1600
Met Gly Trp Asp Asp Trp Ile Ile Ala Pro Leu Glu Tyr Glu Ala Phe
            415                 420                 425

cac tgc gag ggg ctg tgc gag ttc cca ttg cgc tcc cac ctg gag ccc    1648
His Cys Glu Gly Leu Cys Glu Phe Pro Leu Arg Ser His Leu Glu Pro
            430                 435                 440

acg aat cat gca gtc atc cag acc ctg atg aac tcc atg gac ccc gag    1696
Thr Asn His Ala Val Ile Gln Thr Leu Met Asn Ser Met Asp Pro Glu
        445                 450                 455

tcc aca cca ccc acc tgc tgt gtg ccc acg cgg ctg agt ccc atc agc    1744
Ser Thr Pro Pro Thr Cys Cys Val Pro Thr Arg Leu Ser Pro Ile Ser
460                 465                 470                 475

atc ctc ttc att gac tct gcc aac aac gtg gtg tat aag cag tat gag    1792
Ile Leu Phe Ile Asp Ser Ala Asn Asn Val Val Tyr Lys Gln Tyr Glu
                    480                 485                 490
```

gac atg gtc gtg gag tcg tgt ggc tgc agg tag cagcactggc cctctgtctt   1845
Asp Met Val Val Glu Ser Cys Gly Cys Arg
　　　　　　　　495　　　　　　　　　500

cctgggtggc acatcccaag agcccttcc tgcactcctg gaatcacaga ggggtcagga. 1905

agctgtggca ggagcatcta cacagcttgg gtgaaaggg attccaataa gcttgctcgc   1965

tctctgagtg tgacttgggc taaaggcccc cttttatcca caagttcccc tggctgagga   2025

ttgctgcccg tctgctgatg tgacoagtgg caggcacagg tccaggagaa cagactctga   2085

atgggactga gtcccaggaa acagtgcttt ccgatgagac tcagcccacc atttctcctc   2145

acctgggcct tctcagcctc tggactatcc taagcacctc tcaggagagc cacaggtgcc   2205

actgcctcct caaatcacat ttgtgcctga tgacttcctg tccctgggac agttgagaag   2265

ctgactgggc aagagtggga gagaagagga gagggcttga atagagttga ggagtgtgag   2325

gctgttagac tgttagattt aaatgtatat tgatgagcta aaaagacaaaa ctgtgcct   2383

<210> 26
<211> 501
<212> PRT
<213> Homo sapiens

<400> 26

Met Arg Leu Pro Lys Leu Leu Thr Phe Leu Leu Trp Tyr Leu Ala Trp
1　　　　　　　5　　　　　　　10　　　　　　　15

Leu Asp Leu Glu Phe Ile Cys Thr Val Leu Gly Ala Pro Asp Leu Gly
　　　　　　20　　　　　　　25　　　　　　　30

Gln Arg Pro Gln Gly Thr Arg Pro Gly Leu Ala Lys Ala Glu Ala Lys
        35   40   45

Glu Arg Pro Pro Leu Ala Arg Asn Val Phe Arg Pro Gly Gly His Ser
    50   55   60

Tyr Gly Gly Gly Ala Thr Asn Ala Asn Ala Arg Ala Lys Gly Gly Thr
65   70   75   80

Gly Gln Thr Gly Gly Leu Thr Gln Pro Lys Lys Asp Glu Pro Lys Lys
                85   90   95

Leu Pro Pro Arg Pro Gly Gly Pro Glu Pro Lys Pro Gly His Pro Pro
            100   105   110

Gln Thr Arg Gln Ala Thr Ala Arg Thr Val Thr Pro Lys Gly Gln Leu
        115   120   125

Pro Gly Gly Lys Ala Pro Pro Lys Ala Gly Ser Val Pro Ser Ser Phe
    130   135   140

Leu Leu Lys Lys Ala Arg Glu Pro Gly Pro Pro Arg Glu Pro Lys Glu
145   150   155   160

Pro Phe Arg Pro Pro Pro Ile Thr Pro His Glu Tyr Met Leu Ser Leu
                165   170   175

Tyr Arg Thr Leu Ser Asp Ala Asp Arg Lys Gly Gly Asn Ser Ser Val
            180   185   190

Lys Leu Glu Ala Gly Leu Ala Asn Thr Ile Thr Ser Phe Ile Asp Lys
        195   200   205

Gly Gln Asp Asp Arg Gly Pro Val Val Arg Lys Gln Arg Tyr Val Phe
    210   215   220

Asp Ile Ser Ala Leu Glu Lys Asp Gly Leu Leu Gly Ala Glu Leu Arg
225   230   235   240

Ile Leu Arg Lys Lys Pro Ser Asp Thr Ala Lys Pro Ala Ala Pro Gly
            245   250   255

Gly Gly Arg Ala Ala Gln Leu Lys Leu Ser Ser Cys Pro Ser Gly Arg
        260   265   270

Gln Pro Ala Ser Leu Leu Asp Val Arg Ser Val Pro Gly Leu Asp Gly
        275   280   285

Ser Gly Trp Glu Val Phe Asp Ile Trp Lys Leu Phe Arg Asn Phe Lys
    290   295   300

Asn Ser Ala Gln Leu Cys Leu Glu Leu Glu Ala Trp Glu Arg Gly Arg
305   310   315   320

Ala Val Asp Leu Arg Gly Leu Gly Phe Asp Arg Ala Ala Arg Gln Val
325   330   335

His Glu Lys Ala Leu Phe Leu Val Phe Gly Arg Thr Lys Lys Arg Asp
340   345   350

Leu Phe Phe Asn Glu Ile Lys Ala Arg Ser Gly Gln Asp Asp Lys Thr
355   360   365

Val Tyr Glu Tyr Leu Phe Ser Gln Arg Arg Lys Arg Arg Ala Pro Leu
370   375   380

Ala Thr Arg Gln Gly Lys Arg Pro Ser Lys Asn Leu Lys Ala Arg Cys
385   390   395   400

Ser Arg Lys Ala Leu His Val Asn Phe Lys Asp Met Gly Trp Asp Asp
405   410   415

Trp Ile Ile Ala Pro Leu Glu Tyr Glu Ala Phe His Cys Glu Gly Leu
420   425   430

Cys Glu Phe Pro Leu Arg Ser His Leu Glu Pro Thr Asn His Ala Val
435   440   445

Ile Gln Thr Leu Met Asn Ser Met Asp Pro Glu Ser Thr Pro Pro Thr
450   455   460

Cys Cys Val Pro Thr Arg Leu Ser Pro Ile Ser Ile Leu Phe Ile Asp
465   470   475   480

Ser Ala Asn Asn Val Val Tyr Lys Gln Tyr Glu Asp Met Val Val Glu
                485   490   495

Ser Cys Gly Cys Arg
            500

<210> 27
<211> 1378
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (276)..(1130)

<400> 27
ggtagcagca tccacoggge aggaggtcgg aggcagcagg gccttaaagg ctactgagtg   60

cgccggccgt tccgtgtcca gaacctoccc tactcctccg ccttctatta cttggccgca   120

cacogccaag ttccgactcc ggttttcgcc tttgcaaagc ctaaggagga ggttaggaac   180

agcagcgccc ccctccctgc ggccgccgcc ccctgcctct cggctctgct ccctgccgcg   240

tgcgcctggg ccgtgcgccc cggcaggcgc cagcc atg tcg atg ctg ccg tcg   293
                                       Met Ser Met Leu Pro Ser
                                        1   5

```
ttt ggc ttt acg cag gag caa gtg gcg tgc gtg tgc gag gtt ctg cag      341
Phe Gly Phe Thr Gln Glu Gln Val Ala Cys Val Cys Glu Val Leu Gln
         10              15              20

caa ggc gga aac ctg gag cgc ctg ggc agg ttc ctg tgg tca ctg ccc      389
Gln Gly Gly Asn Leu Glu Arg Leu Gly Arg Phe Leu Trp Ser Leu Pro
     25              30              35

gcc tgc gac cac ctg cac aag aac gag agc gta ctc aag gcc aag gcg      437
Ala Cys Asp His Leu His Lys Asn Glu Ser Val Leu Lys Ala Lys Ala
 40              45              50

gtg gtc gcc ttc cac cgc ggc aac ttc cgt gag ctc tac aag atc ctg      485
Val Val Ala Phe His Arg Gly Asn Phe Arg Glu Leu Tyr Lys Ile Leu
55              60              65              70

gag agc cac cag ttc tcg cct cac aac cac cca aaa ctg cag caa ctg      533
Glu Ser His Gln Phe Ser Pro His Asn His Pro Lys Leu Gln Gln Leu
             75              80              85

tgg ctg aag gcg cat tac gtg gag gcc gag aag ctg cgc ggc cga ccc      581
Trp Leu Lys Ala His Tyr Val Glu Ala Glu Lys Leu Arg Gly Arg Pro
         90              95              100

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cgc acc atc tgg gac ggc gag gag acc agc tac tgc ttc aag gag aag      677
Arg Thr Ile Trp Asp Gly Glu Glu Thr Ser Tyr Cys Phe Lys Glu Lys
 120             125             130

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Ser Arg Gly Val Leu Arg Glu Trp Tyr Ala His Asn Pro Tyr Pro Ser
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Gln Val Ser Asn Trp Phe Lys Asn Arg Arg Gln Arg Asp Arg Ala Ala
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            185                 190                 195

aag cag aac caa ctc tct cct ctg gaa ggg ggc aag ccg ctc atg tcc        917
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agc tca gaa gag gaa ttc tca cct ccc caa agt cca gac cag aac tcg        965
Ser Ser Glu Glu Glu Phe Ser Pro Pro Gln Ser Pro Asp Gln Asn Ser
215                 220                 225                 230

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Val Leu Leu Leu Gln Gly Asn Met Gly His Ala Arg Ser Ser Asn Tyr
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tct ctc ccg ggc tta aca gcc tcg cag ccc agt cac ggc ctg cag acc       1061
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His Gln His Gln Leu Gln Asp Ser Leu Leu Gly Pro Leu Thr Ser Ser
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Leu Val Asp Leu Gly Ser
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Pro Lys Leu Gln Gln Leu Trp Leu Lys Ala His Tyr Val Glu Ala Glu
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Lys Leu Arg Gly Arg Pro Leu Gly Ala Val Gly Lys Tyr Arg Val Arg
                100   105   110

Arg Lys Phe Pro Leu Pro Arg Thr Ile Trp Asp Gly Glu Glu Thr Ser
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Tyr Cys Phe Lys Glu Lys Ser Arg Gly Val Leu Arg Glu Trp Tyr Ala
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Gly Lys Pro Leu Met Ser Ser Ser Glu Glu Glu Phe Ser Pro Pro Gln
    210   215   220

Ser Pro Asp Gln Asn Ser Val Leu Leu Leu Gln Gly Asn Met Gly His
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           20              25              30

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Ser Ser Gly Ser Asp Glu Lys Pro Cys Arg Val His Ala Ala Arg Cys
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Gly Leu Gln Gly Ala Arg Arg Arg Ala Gly Gly Arg Arg Ala Gly Gly
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Gly Gly Pro Gly Gly Arg Pro Gly Arg Glu Pro Arg Gln Arg His Thr
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tcc aag att gag acg ctg cgc ctg gcc tcc agc tac atc tcg cac ctg      926
Ser Lys Ile Glu Thr Leu Arg Leu Ala Ser Ser Tyr Ile Ser His Leu
         115               120               125

ggc aac gtg ctg ctg gcg ggc gag gcc tgc ggc gac gga cag ccc tgc      974
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     145               150               155

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160               165               170               175

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Leu Gln Gly Ala Arg Arg Arg Ala Gly Gly Arg Arg Ala Gly Gly Gly
    50   55   60

Gly Pro Gly Gly Arg Pro Gly Arg Glu Pro Arg Gln Arg His Thr Ala
65   70   75   80

Asn Ala Arg Glu Arg Asp Arg Thr Asn Ser Val Asn Thr Ala Phe Thr
           85   90   95

Ala Leu Arg Thr Leu Ile Pro Thr Glu Pro Ala Asp Arg Lys Leu Ser
           100   105   110

Lys Ile Glu Thr Leu Arg Leu Ala Ser Ser Tyr Ile Ser His Leu Gly
          115   120   125

Asn Val Leu Leu Ala Gly Glu Ala Cys Gly Asp Gly Gln Pro Cys His
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Ser Gly Pro Ala Phe Phe His Ala Ala Arg Ala Gly Ser Pro Pro Pro
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Pro Pro Pro Pro Pro Pro Ala Arg Asp Gly Glu Asn Thr Gln Pro Lys
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Gln Ile Cys Thr Phe Cys Leu Ser Asn Gln Arg Lys Leu Ser Lys Asp
         180   185   190

Arg Asp Arg Lys Thr Ala Ile Arg Ser
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KALE S ET AL: "THREE-DIMENSIONAL CELLULAR DEVELOPMENT IS ESSENTIAL FOR EX VIVO FORMATION OF HUMAN BONE" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 18, September 2000 (2000-09), pages 954-958, XP000996174 ISSN: 1087-0156 the whole document -/--	1-30, 32-34, 42-44, 46,51, 54,55, 58-60, 62-64, 68-85, 88, 92-96, 149-160

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 December 2004	05/01/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chavanne, F

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/30742 A (BIOSURFACE TECH INC) 16 November 1995 (1995-11-16) abstract page 20 - page 29 page 30, line 12 - page 31, line 7 page 32, line 28 - page 33, line 31 page 36, line 30 - page 40 examples 3,4	1-30, 32-34, 42-48, 51-60, 62-86, 88-112, 114-141, 149-160
X	KUSHIDA A, ET AL.: JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, vol. 45, no. 5, 1999, pages 355-362, XP001204072 abstract page 355, column 2 - page 356, column 1, paragraph 1 page 356, column 2, paragraph 2 page 357, column 2, paragraph 2 figure 1 page 359 page 360, column 1, paragraph 2 page 360, column 2, paragraph 2 - page 361, column 1, paragraph 1	1,3-12, 14-23, 26-30, 34-36, 42,43, 48,49, 51-53, 62-64, 74-79, 81-85
X	WO 95/33821 A (ADVANCED TISSUE SCIENCES INC) 14 December 1995 (1995-12-14) abstract page 15, paragraph 2 - page 17, paragraph 1 page 18, paragraph 7 page 19, paragraph 2 - page 30, paragraph 2 page 45, paragraph 1 - page 46, paragraph 1 ---/---	1-16, 18-30, 32-41, 43-86, 88-112, 114-160

3

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/091979 A1 (ESCHENHAGEN THOMAS) 15 May 2003 (2003-05-15) abstract paragraphs '0001!, '0008!, '0014!, '0016!, '0031!, '0083! ~ '0085! example 2 —————	1-6,16, 18-31, 74-77, 85-87, 91-94, 96,149, 150,154, 156,157

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9530742	A	16-11-1995	AU	2435495 A	29-11-1995
			AU	2468995 A	29-11-1995
			WO	9530383 A1	16-11-1995
			WO	9530742 A1	16-11-1995
			US	5723331 A	03-03-1998
			US	5786217 A	28-07-1998
WO 9533821	A	14-12-1995	US	5902741 A	11-05-1999
			AU	689605 B2	02-04-1998
			AU	2769695 A	04-01-1996
			CA	2192064 A1	14-12-1995
			EP	0812351 A1	17-12-1997
			JP	2002502226 T	22-01-2002
			NZ	288467 A	28-10-1998
			WO	9533821 A1	14-12-1995
			US	5962325 A	05-10-1999
			US	6140039 A	31-10-2000
			US	6022743 A	08-02-2000
			US	5863531 A	26-01-1999
US 2003091979	A1	15-05-2003	DE	10003521 A1	09-08-2001
			AU	3734501 A	07-08-2001
			CA	2398084 A1	02-08-2001
			WO	0155297 A2	02-08-2001
			EP	1250416 A2	23-10-2002
			JP	2004500093 T	08-01-2004

| Box No. I | Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet) |

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

    a. type of material

      [X] a sequence listing

      [ ] table(s) related to the sequence listing

    b. format of material

      [X] in written format

      [X] in computer readable form

    c. time of filing/furnishing

      [X] contained in the international application as filed

      [X] filed together with the international application in computer readable form

      [ ] furnished subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Box II   Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

    Although claims 97-141 are directed to a method of treatment of the
    human/animal body, the search has been carried out and based on the alleged
    effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III   Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.